Tampere University

VESA LINDSTRÖM

Severe Pneumococcal Infections and Vaccination in Patients with Hematological Malignancy

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ACADEMIC DISSERTATION
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It's not about ideas.
It's about making ideas happen.

Scott Belsky

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Vesa Lindström

ABSTRACT

Patients with hematological malignancies are at high risk for severe infections such as bacteremia, pneumonia, and meningitis caused by *Streptococcus pneumoniae* (invasive pneumococcal disease (IPD)). IPD is related to marked morbidity and mortality in these patients. However, only limited population-based data is available concerning IPD in patients with hematological malignancies.

Pneumococcal vaccines currently in use have restricted efficacy against IPD in patients with hematological malignancies. In patients with chronic lymphocytic leukemia (CLL, a malignancy of mature B-lymphocytes), pneumococcal polysaccharide vaccines have so far been inefficient. In comparison, T-cell dependent conjugate vaccines are more immunogenic and are capable of inducing immunologic memory. It has been shown that some CLL patients achieve protective antibody responses against IPD with conjugate vaccines.

The aims of this thesis were to evaluate the incidence of IPD, the serotype distribution of the isolated strains, and serotype coverage of pneumococcal vaccines in Finnish patients with specific hematological malignancies. In CLL patients, the main objectives were to assess the antibody persistence after five years of the administration of 7-valent pneumococcal conjugate vaccine and the efficacy of the 23-valent pneumococcal polysaccharide vaccine booster dose given five years after the conjugate vaccine. Furthermore, the activation and expression of indoleamine 2,3-dioxygenase (IDO), which suppresses T-cell functions by catalyzing tryptophan metabolism, was evaluated, including its possible effects on antibody response to 7-valent pneumococcal conjugate vaccine.

The overall incidence rate of IPD among patients with hematological malignancies was 3.8 per 1000 person-years within one year of a diagnosis of hematological malignancy. The highest rate of IPD was in patients with multiple myeloma. The risk of IPD in patients with multiple myeloma was 2.7 to 12 times higher compared to other hematological malignancies. The most common serotypes causing IPD were 14 and 6B. The serotype distribution was, to some extent, different from the general adult population. In CLL patients, 57% and 64% of isolates were covered by 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal

polysaccharide vaccine, respectively, while 32% of isolates were covered by neither of these vaccines.

The antibody concentrations in 24 CLL patients and 8 controls five years after the administration of 7-valent pneumococcal conjugate vaccine did not differ statistically. A trend toward lower concentrations was seen in CLL patients for six serotypes compared to controls. In CLL patients, median antibody concentrations declined almost 50% for four serotypes over the course of five years following the administration of 7-valent pneumococcal conjugate vaccine.

Only 10 to 15% of the CLL patients achieved a significant response to pneumococcal antigens included in 7-valent pneumococcal conjugate vaccine one month after the administration of 23-valent pneumococcal polysaccharide booster vaccine defined as an at least two-fold increase and a post-vaccination concentration of at least 0.35 μg/ml. Antibody concentrations were significantly higher in controls for four antigens included in the conjugate vaccine and for both measured antigens in the polysaccharide vaccine.

IDO activity and expression was assessed in 49 CLL patients and 24 controls. IDO activity was significantly higher in patients with CLL. In contrast, IDO expression in peripheral blood mononuclear cells representing malignant B-cells was significantly reduced. Thus, these findings suggest that increased IDO activation is derived from cells other than malignant B-cells. Increased IDO activity did not have an effect on vaccine responses.

In conclusion, serotype coverage of pneumococcal vaccines in patients with hematological malignancies seems to be lower compared to general population. In patients with CLL, pneumococcal conjugate vaccine given at an early stage of the disease seems to induce an antibody response lasting at least five years. However, pneumococcal conjugate vaccine is not capable of inducing properly functional memory B-cells in CLL patients. Hence, 23-valent pneumococcal polysaccharide vaccine given after conjugate vaccine does not seem to bring any benefits in vaccination strategies in patients with CLL.

TIIVISTELMÄ

Hematologista maligniteettia sairastavien potilaiden riski sairastua *Streptococcus pneumoniaen* aiheuttamiin vakaviin invasiivisiin pneumokokki-infektioihin (IPI) on suuri. Näitä infektioita ovat sepsis, keuhkokuume ja aivokalvontulehdus. IPI:in liittyy tässä korkean riskin potilasryhmässä huomattava sairastavuus ja kuolleisuus. Hematologista maligniteettia sairastavien potilaiden IPI:a koskevaa väestöpohjaista tutkimustietoa on vähän.

Nykyään käytettävien pneumokokkirokotteiden teho IPI:a vastaan hematologista maligniteettia sairastavilla on alentunut. Kroonista lymfaattista leukemiaa (KLL, kypsien B-lymfosyyttien pahanlaatuinen tauti) sairastavilla potilailla pneumokokkipolysakkaridirokotteet ovat toistaiseksi osoittautuneet tehottomiksi. T-soluista riippuvaiset konjugaattirokotteet ovat immunogeeniselta teholtaan parempia ja pystyvät muodostamaan immunologisen muistin. Osalle KLL-potilaista konjugaattirokotteet tuottavat suojaavan vasta-ainevasteen IPI:a vastaan.

Väitöskirjatyön tavoitteena oli arvioida suomalaisilla hematologista maligniteettia sairastavilla potilailla IPI:n ilmaantuvuutta ja serotyyppijakaumaa sekä pneumokokkirokotteiden serotyyppikattavuutta. KLL-potilailla tavoitteena oli selvittää pneumokokkivasta-aineiden säilymistä seitsemän serotyypin konjugaattirokotuksen jälkeen sekä arvioida 23 serotyypin polysakkaridirokotteen tehoa, kun tehosteannos annettiin viiden vuoden kuluttua konjugaattirokotteesta. Lisäksi selvitettiin tryptofaania katalysoivan ja T-solujen toimintaa heikentävän indoleamiini 2,3-dioksigenaasi -entsyymin (IDO) aktivaatiota ja ekspressiota sekä sen mahdollista vaikutusta seitsemän serotyypin konjugaattirokotteen rokotevasteeseen KLL-potilailla.

IPI:n ilmaantuvuus yhden vuoden kuluessa hematologisen maligniteetin diagnoosista oli 3.8 per 1000 henkilövuotta. Suurin ilmaantuvuus oli multippelia myeloomaa sairastavilla potilailla, joiden riski sairastua IPI:iin oli 2.7-12 kertainen muihin hematologisiin maligniteetteihin verrattuna. Yleisimmät IPI-tapausten pneumokokkiserotyypit olivat 14 ja 6B. Serotyyppien jakauma erosi hieman muun aikuisväestön serotyyppijakaumasta. Kolmentoista serotyypin konjugaattirokote kattoi 57% IPI:n aiheuttaneista kannoista ja 23 serotyypin polysakkaridirokote 64%. Kumpikaan rokote ei kattanut 32 % kannoista.

Viiden vuoden kuluttua seitsemän serotyypin konjugaattirokotuksesta ei todettu tilastollisesti merkittävää eroa 24 KLL-potilaan ja kahdeksan verrokkipotilaan pneumokokkivasta-ainepitoisuuksissa. Kuutta serotyyppiä kohtaan vasta-ainepitoisuudet olivat KLL-potilailla matalampia kuin verrokkipotilailla. Näistä neljää serotyyppiä kohtaan vasta-ainepitoisuudet laskivat lähes 50%.

Kahdenkymmenenkolmen serotyypin polysakkariditehosterokotteella vain 10-15% KLL-potilaista sai merkittävän rokotevasteen seitsemän serotyypin konjugaattirokotteen sisältämiä serotyyppejä kohtaan. Verrokkipotilaiden vasta-ainepitoisuudet olivat merkitsevästi korkeampia neljälle konjugaattirokotteen ja kahdelle mitatulle polysakkaridirokotteen sisältämille serotyypeille.

IDO:n aktiivisuus ja ekspressio määritettiin 49 KLL-potilaalta ja 24 verrokilta. KLL-potilaiden IDO:n aktiivisuus oli merkitsevästi korkeampi kuin verrokeilla. IDO:n ekspressio pahanlaatuisia B-soluja edustavissa veren mononukleaarisissa soluissa oli KLL-potilailla kuitenkin merkitsevästi alentunut. Tämä viittaa siihen, että KLL-potilaiden suurentunut IDO:n aktiivisuus on peräisin muista soluista kuin pahanlaatuisista B-soluista. Rokotevasteisiin lisääntyneellä IDO:n aktiivisuudella ei havaittu olevan vaikutusta.

Yhteenvetona, pneumokokkirokotteiden serotyyppikattavuus hematologista maligniteettia sairastavilla potilailla vaikuttaa olevan matalampi kuin muulla väestöllä. Pneumokokkikonjugaattirokotteen avulla suojaavan rokotusvasteen invasiivista pneumokokki-infektiota vastaan saaneiden KLL-potilaiden vasta-aineiden todettiin säilyvän vähintään viiden vuoden ajan. KLL-potilaat eivät kuitenkaan pysty muodostamaan normaalisti toimivia muisti B-soluja konjugaattirokotteen turvin. Näin ollen 23 serotyypin pneumokokkipolysakkariditehosterokote ei ole hyödyllinen osana KLL-potilaiden rokotusohjelmaa.

CONTENTS

ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ATM	Ataxia telangiectasia mutated
BCL-2	B-cell lymphoma-2
BCR	B cell receptor
BIRC3	Baculoviral IAP repeat-containing protein 3
Btk	Bruton's tyrosine kinase
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CIT	Chemoimmunotherapy
CLL	Chronic lymphocytic leukemia
COX-2	Cyclooxygenase-2
CRM	Cross-reacting material
CSF	Cerebrospinal fluid
Ct	Threshold cycle
DC	Dendritic cell
EBMT	European Society for Blood and Marrow Transplantation
ECIL	European Conference on Infectious in Leukaemia
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FISH	Fluorescence in situ hybridization
GMC	Geometric mean concentration
GM-CSF	Granulocyte-macrophage-colony-stimulating factor
HIV	Human immunodeficiency virus
HSCT	Hematopoietic stem cell transplantation
ICD	International Statistical Classification of Diseases and Related Health Problems
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
Ig	Immunoglobulin

IGHV	Immunoglobulin heavy chain variable gene
IGHV-M	Mutated immunoglobulin heavy chain variable gene
IGHV-UM	Unmutated immunoglobulin heavy chain variable gene
IL	Interleukin
IPD	Invasive pneumococcal disease
KYN	Kynurenine
mAb	Monoclonal antibody
MDS	Myelodysplastic syndrome
MYD88	Myeloid differentiation primary response 88
NCL	Nurse-like cell
NIDR	The National Infectious Disease Register
NOTCH1	Notch homolog 1, translocation-associated (Drosophila)
NVT	Non-vaccine serotype
OPA	Opsonophagocytic assay
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PCV7	7-valent pneumococcal conjugate vaccine
PCV10	10-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PD-1	Programmed cell death protein 1
PI3K	Phosphaditylinositol-3 kinase
PPV	Pneumococcal polysaccharide vaccine
PPV23	23-valent pneumococcal polysaccharide vaccine
PS	Polysaccharide
RNA	Ribonucleic acid
RQ	Relative quantification
SF3B1	Splicing factor 3B subunit 1
Syk	Spleen tyrosine kinase
THL	The National Institute for Health and Welfare
TNF-α	Tumor necrosis factor alpha
TP53	Tumor protein P53
Treg	Regulatory T-cell
TRP	Tryptophan
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to in the text by Roman numerals I-IV:

I Lindström V, Aittoniemi J, Jylhävä J, Eklund C, Hurme M, Paavonen T, Oja SS, Itälä-Remes M, Sinisalo M (2012). Indoleamine 2,3-dioxygenase activity and expression in patients with chronic lymphocytic leukemia. Clin Lymphoma Myeloma Leuk 12: 363-365.

II Lindström V, Aittoniemi J, Lyytikäinen O, Klemets P, Ollgren J, Silvennoinen R, Nuorti JP, Sinisalo M (2016). Invasive pneumococcal disease in patients with haematological malignancies before routine use of conjugate vaccines in Finland. Infect Dis 48: 399-402.

III Lindström V, Aittoniemi J, Salmenniemi U, Käyhty H, Huhtala H, Itälä-Remes M, Sinisalo M (2018). Antibody persistence after pneumococcal conjugate vaccination in patients with chronic lymphocytic leukemia. Hum Vaccin Immunother 14: 1471-1474.

IV Lindström V, Aittoniemi J, Salmenniemi U, Käyhty H, Huhtala H, Sinisalo M (2019). Antibody response to the 23-valent pneumococcal polysaccharide vaccine after conjugate vaccine in patients with chronic lymphocytic leukemia. Hum Vaccin Immunother (Epub ahead of print).

1 INTRODUCTION

Invasive pneumococcal disease (IPD) is caused by Gram-positive bacteria *Streptococcus pneumoniae* spreading to normally sterile sites leading to bacteremia, pneumonia, or meningitis (Drijkoningen & Rohde 2014). Immunocompromised persons, such as patients with hematological malignancies are at the highest risk for IPD (Kyaw et al. 2005). Only limited population-based data are available concerning IPD in these patients. In a previous study, adults with hematological malignancies had a 50 times higher risk for IPD compared to the general population (Klemets et al. 2008).

Chronic lymphocytic leukemia (CLL) is a malignancy of mature B-cell lymphocytes. CLL is the most common type of leukemia in adult Caucasians (Chiorazzi et al. 2005). Infections are the most common cause of mortality in CLL patients. The majority of infections are caused by common bacterial pathogens, including *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae* (Morrison 2010). Patients with CLL have significant dysfunctions of the immune system predisposing to severe infections, particularly in the advanced stage of the disease (Dearden 2008; Forconi & Moss 2015).

Antibody response particularly to pneumococcal polysaccharide vaccines (PPVs) has been poor in patients with CLL and other hematological malignancies (Hartkamp et al. 2001; Hinge et al. 2012; Sinisalo et al. 2001). Pneumococcal conjugate vaccines (PCVs) have been shown to induce better, albeit still limited, antibody responses in these patients (Cordonnier et al 2009; Sinisalo et al. 2007; Svensson et al. 2018).

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyzes the degradation of tryptophan (trp) into kynurenine (kyn). IDO suppresses T-cell proliferation and activation by reducing trp concentration in tissues contributing to immunotolerance and immunosuppression (Mellor & Munn 1999). Overexpression of IDO has been shown in many malignant diseases in which it diminishes the response of the immune system against the tumor cells (Munn & Mellor 2016).

The aim of this thesis was to distinguish features of IPD in patients with hematological malignancies and evaluate serotype coverage of pneumococcal

vaccines. In CLL patients, antibody persistence after PCV and the efficacy of the PPV booster after PCV were assessed. Furthermore, IDO activation and expression and the possible effect on vaccine response were determined.

2 REVIEW OF THE LITERATURE

2.1 Invasive pneumococcal disease (IPD)

2.1.1 Streptococcus pneumoniae

Streptococcus pneumoniae is a Gram-positive bacteria, the virulence of which is mainly derived from external polysaccharide capsules and cholesterol-dependent toxin pneumolysin (Briles et al. 1992; Hyams et al. 2010; Kadioglu et al. 2008). Today, almost 100 distinct capsular types (serotypes) have been described (Geno et al. 2015). *S.pneumoniae* serotypes can cause asymptomatic colonization in nasopharynx of about 5% of adults over 50 years of age (Le Polain de Waroux et al. 2014). When spreading from the nasopharynx, serotypes can cause mucosal infections like otitis, sinusitis, and bronchitis (Sorensen & Edgar, 2018). Only 20 to 30 serotypes can produce significant pneumococcal infection (Geno et al. 2015).

2.1.2 Definition and epidemiology

When *S.pneumoniae* serotypes invade normally sterile sites they can cause bacteremia, pneumonia, and meningitis. These are defined as invasive pneumococcal disease (IPD) (Bogaert et al. 2004). Globally IPD has a major impact on morbidity and mortality (Drijkoningen and Rohde 2014; Verhaegen et al. 2014). Incidence of IPD is influenced by e.g. age, socioeconomic factors, and comorbidities (Chapman et al. 2013; Klemets et al. 2008; Reinert et al. 2005). Depending on the age, young children and older adults are at the highest risk for IPD (Pérez-Trallero et al. 2009; Harboe et al. 2010). During the 1980s and 1990s, before the introduction of 7-valent pneumococcal conjugate vaccine (PCV7) to the childhood vaccination program, the median incidence of IPD in Denmark was 15.7 per 100 000 (Harboe et al. 2010). After the introduction of pneumococcal conjugate vaccines (PCVs), the incidence of IPD has mainly declined due to indirect herd immunity (Rashid et al. 2012). After 3 years of childhood PCV vaccination program in Denmark, the overall incidence

rate of IPD decreased significantly from 19.5 to 17.7 per 100 000. A corresponding decrease in IPD incidence in adults 65 years of age and older was from 65.5 to 59.7 per 100 000 (Ingels et al. 2012). In the United States, the incidence rate of IPD in adults 50 years of age and older declined from 40.8 cases/100 000 in 1998-1999 to 29.4/100 000 in 2002-2003, i.e. 28% (Lexau et al. 2005). In Finland, the use of 10-valent pneumococcal conjugate vaccine (PCV10) in infant vaccination program decreased pneumonia hospitalizations by 6.7% in persons aged over 65 years (Okasha et al. 2018). A corresponding decline in IPD incidence has been reported also with the use of 13-valent pneumococcal conjugate vaccine (PCV13) (Camara et al. 2017; Harboe et al. 2014; Moore et al. 2015). Although the incidence of IPD in adults 65 years of age and older has declined since the introduction of PCV13, it still remains high (Harboe et al. 2014). A reduction in the IPD incidence has usually been seen for those serotypes which are included in conjugate vaccines whereas the incidence rate due to non-vaccine serotypes (NVTs) has increased. This phenomenon is called serotype replacement (van der Linden et al. 2015). Contrary, in Ireland the overall incidence rate of IPD in adults has not changed, but the incidence rate due to PCV serotypes has also declined (Corcoran et al. 2017). Furthermore, in Sweden in adults over 65 years of age, the proportion of NVTs increased after the introduction of PCVs, and they now account for 72% of IPD cases (Naucler et al. 2017). Recent analysis from 10 European countries consistently revealed that the incidence rates of IPD caused by PCV serotypes declined in adults over 65 years of age by more than 70% over a 5 year period of childhood vaccination programs with PCV10 or PCV13. An increase in NVTs only accounted for 9% of the overall decline (Hanquet et al. 2018).

The serotype distribution in IPD has changed over the decades. In Denmark, before the introduction of PCV7, the proportion of serotypes 4, 9, and 19A increased, proportion of serotype 18C decreased, and serotype 2 almost disappeared (Harboe et al. 2010). During the first 3 years of the PCV7 vaccination program in the overall Danish population, the proportions of NVTs 7F and 19A increased significantly (Ingels et al. 2012). The proportion of serotype 19A also increased during the first 2 years after the introduction of PCV13 in adults 65 years of age and older (Harboe et al. 2014).

2.1.3 IPD in hematological malignancies

2.1.3.1 Incidence and risk

Patients with hematological malignancies are at the highest risk of IPD (Breiman et al. 1997; Kyaw et al. 2005). The epidemiologic data on IPD in specific hematological malignancies are still quite limited. In two surveillance programs in the United States, the incidence rate of IPD in adults with hematologic cancer was 503 per 100 000 persons, which was the highest in adults with chronic illnesses (Kyaw et al. 2005). A comparable risk was found in the Finnish population where the incidence rate of IPD in patients with hematological malignancies was 547 per 100 000 persons, which was higher than in other immunocompromising conditions (Klemets et al. 2008). The risk of IPD is estimated to be highest in patients with multiple myeloma, a malignancy of plasma cells (Mufson et al. 2012; Savage et al. 1982; Wong et al. 2010). The risk of IPD in myeloma patients in the Canadian province of Alberta from 2000 to 2004 was over 60 times higher than in the general population (Wong et al. 2010). The risk was even higher in the Gothenburg region in Sweden between 1996 and 2008 where myeloma patients had 154 times higher risk for IPD than persons without myeloma (Backhaus et al. 2016).

There are only few studies available concerning incidence rates of IPD in patients with CLL. A study from Alberta revealed an incidence rate of 124 per 100 000 patients, which led to 12.6-fold risk for IPD in patients with CLL compared to the general population (Wong et al. 2010). A higher incidence rate was found in the Gothenburg area in Sweden, where the rate was 429 per 100 000 patients. The risk for IPD was 29-fold compared with persons without CLL (Backhaus et al. 2016).

The data from patients with acute leukemia are even more limited. In a study of a pediatric acute lymphoblastic leukemia (ALL) population, the risk for IPD varied between age groups from 7.6 to 50.6 -fold compared with the general population (Meisel et al. 2007). In Alberta, the incidence rate of IPD in adults 18 years of age and older with acute myeloid or lymphoblastic leukemia was 129 per 100 000 patients. The risk for IPD was 11.9-fold compared to the general population (Wong et al. 2010).

2.1.3.2 Serotype distribution

In a study with adult patients suffering from hematological malignancies and lung cancer, the only serotype with an increased prevalence compared to the general adult population was serotype 6A (Wong et al. 2010). This serotype was independently related to hematological malignancies in a study with immunocompromised adults. Serotypes 10A, 11A, 23F, and 33F were also more frequently found in the study population on the whole (Luján et al. 2013). In a retrospective study in two French hematology departments, the most frequent serotypes were 9V and 19F, but only 25 IPD episodes were observed (Debbache et al. 2009). In the European Society for Blood and Marrow Transplantation (EBMT) survey, the serotypes identified in 51 IPD episodes after hematopoietic stem cell transplantation (HSCT), were 6, 9, 11, 14, 22, and 23F, but only 33% of the episodes were serotyped (Engelhard et al. 2002). Serotype replacement in patients with hematological malignancy after the introduction of PCVs has not been studied. However, rates of IPD among adults with HIV (human immunodeficiency virus), who represent another important group of immunocompromised persons, non-PCV7 serotypes increased 60% during the 7 years after PCV7 introduction (Cohen et al. 2010).

2.2 Chronic lymphocytic leukemia (CLL)

2.2.1 Background

CLL is a malignancy of mature B lymphocytes (Chiorazzi et al. 2005). It is characterized by an expansion of clonal B lymphocytes which proliferates in the bone marrow, peripheral blood, lymph nodes, and spleen. CLL is the most common type of adult leukemia in Caucasians, accounting for almost 40% of leukemias (Linet et al. 2007). The count of peripheral blood B lymphocytes $\geq 5 \times 10^9/l$, lasting for at least 3 months, is required for diagnosis of CLL. Diagnosis is confirmed from peripheral blood by flow cytometry, evincing clonality of B lymphocytes expressing either κ or λ immunoglobulin light chains. Furthermore, the typical immunophenotypic features of CLL include the expression of CD19, CD5, CD20, and CD23 expressions (Rawstron et al. 2018).

During the normal maturation, B lymphocytes undergo rearrangement of immunoglobulin (Ig) variable (V) genes. This rearrangement encompasses mutations in V_HDJ_H and V_LJ_L gene segments (Chiorazzi et al. 2005). On the basis of the heavy

chain variable region gene mutations, CLL is divided into two groups: one where CLL cells have rearranged V_H genes ("mutated" CLL; IGHV-M) and the other one with few or no mutations at all ("unmutated" CLL) (Hashimoto et al. 1995). The unmutated IGHV mutation status has been shown to associate with shorter survival (Hamblin et al. 1999).

Signaling through B-cell receptor (BCR) plays a critical role in the survival and proliferation of CLL clones. Stimulation of BCR by antigen engagement induces activation of downstream kinases spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (Btk), and phosphatidylinositol-3 kinases (PI3Ks) (Herman et al. 2011; Quiroga et al. 2009; Ringshausen et al. 2002). Furthermore, interactions between CLL cells and the bone marrow and/or lymphoid tissue microenvironment are in the key role, promoting the growth and prevention of apoptosis in CLL cells. The most important factors in the CLL microenvironment are monocyte-derived nurse-like cells (NLCs), mesenchymal stromal cells, follicular dendritic cells (DC), endothelial cells, and T-cells (Burger et al. 2009; Ten Hacken & Burger 2016).

2.2.2 Genetic lesions in CLL

CLL is divided into three risk groups based on the fluorescence in situ hybridization (FISH) model: high-risk, intermediate-risk and low-risk CLL. The most frequent genetic lesion found in 50-60% of CLL cases is deletions of the 13q14 region. Del13q14 is more frequently associated with IGHV-M CLL and is usually the only cytogenetic abnormality detected. Patients with del13q14 tend to have favorable prognosis and are categorized as low-risk patients (Dohner et al. 2000).

Trisomy 12, an extra copy of chromosome 12, is found in almost 15% of CLL patients. It has been categorized as intermediate-risk CLL in the past. An unfavorable prognosis is attached to patients with deletions in the 11q22-23 chromosomal region or in the 17p13 region and a mutationally inactivated tumor suppressor gene TP53. 11q deletions are found in almost 20% of advanced stage CLL patients and are frequently connected to the inactivation of the tumor suppressor gene ataxia telangiectasia mutated (ATM) and IGHV-UM CLL type. Marked lymphadenopathy is a common clinical feature of patients with 11q deletions. 17p deletions and TP53 mutations are found in less than 10% of newly diagnosed CLL patients. These patients are usually refractory to traditional chemotherapy and, before the era of new therapeutic regimens, their prognosis was very poor. The proportion of TP53 disruptions increase as CLL progresses (Rossi & Gaidano 2016; Dohner et al. 2000).

Novel relevant molecular mutations identified with next-generation sequencing techniques are NOTCH1, SF3B1, MYD88, and BIRC3. NOTCH1 gene mutations have been found in almost 10% of CLL patients at diagnosis. NOTCH1 mutated patients usually belong to the IGHV-UM group. NOTCH1 mutations are now known to be independent poor prognostic markers in CLL (Rossi et al. 2012). Almost 40% of patients also exhibit trisomy 12 in addition to NOTCH1 mutations. Thus, some trisomy 12 patients have poorer survival than previously assumed (Del Giudice et al. 2012). SF3B1 gene mutations affecting the spliceosome complex are found in almost 10% of CLL cases (Quesada et al. 2011). Mutations in the MYD88 gene occur in less than 5% of patients and been found specifically in the IGHV-M group. Survival in this group has been high, but the final impact on the prognosis is not clear (Martínez-Trillos et al. 2014). Mutations in the BIRC3 gene leading to NF-κβ activation are found in less than in 10% of CLL patients at diagnosis. BIRC3 mutated patients have very poor prognosis. According to a new risk stratification, patients with BIRC3 mutation or TP53 disruptions are categorized as high-risk patients. NOTCH1 and SF3B1 mutated patients are considered as intermediate-risk CLL patients. Patients with 11q deletions are also assigned to this risk group. Patients with trisomy 12 or normal karyotype are considered as low-risk CLL patients and only patients who have del13q14 belong to the very-low risk group (Fabbri & Dalla-Favera 2016; Rossi & Gaidano 2016). So far, in routine clinical practice genetic risk stratification is performed based on FISH and TP53 mutation analysis and the new risk stratification is used in clinical trials.

2.2.3 Immunodeficiency

Patients with CLL have significant disturbances of the immune system from the early stages of the disease (Hamblin & Hamblin 2008). Their innate (humoral) and adaptive (cell-mediated) immune responses have both quantitative and qualitative defects (Aittoniemi et al. 1999; Ravandi & O'Brien 2006). Increased $CD4^+$ and $CD8^+$ counts can be found at an early stage of the disease (Dearden 2008). Chronic viral infections including cytomegalovirus may cause profound changes in T cell counts (Mackus et al. 2003; Pourgheysari et al. 2007). T cells also exhibit functional disturbances. Particularly, T-helper activity is impaired while T-suppressor activity is increased (Dearden 2008). Furthermore, in the advanced stages of the CLL, the proportion of $FoxP3^+$ regulatory T cells (Tregs) is increased (Beyer et al. 2005). Defects in T cell function lead to impaired interaction with B cells (Dearden 2008).

Abnormalities in T cells increase with CLL progression (Forconi & Moss 2015). Defects in neutrophil and monocyte functions and reduced levels and activity of complement proteins have also been described (Itälä et al. 1996; Schlesinger et al. 1996).

A significant feature of immune suppression in CLL is hypogammaglobulinemia. At least one serum immunoglobulin can be diminished at an early stage of the disease (Davey et al. 1987; Hamblin & Hamblin 2008). As the disease progresses, hypogammaglobulinemia becomes more severe and involves all Ig classes (Dearden 2008; Morrison 2010). Mechanisms leading to Ig depletion in CLL are not fully understood. CLL cells resemble recently discovered natural Breg cells and act like anergic B cells reducing production of immunoglobulins by direct cell contact. Furthermore, the level of soluble factors essential for plasma cell survival decreases and production of immunosuppressive IL-10 is increased (Forconi & Moss 2015). A correlation between the degree of hypogammaglobulinemia and bacterial infections and survival has been shown (Dearden 2008; Morrison 2010). In some studies, no differences were detected between Ig levels with regard to the IGHV mutation status (Francis et al. 2006; Sinisalo et al. 2004).

2.2.4 Treatment

Treatment of CLL should be initiated only when there is evidence of progressive or symptomatic disease (Hallek et al. 2018). The Binet classification, where CLL is divided into groups A (early stage of the disease), B (intermediate), or C (advanced stage), depending on the distribution of lymphadenopathy and presence of anemia and/or thrombocytopenia, is still in use in the staging of the disease (Binet et al. 1981). Based on the genetic risk stratification chemoimmunotherapy (CIT), in which chemotherapy agents are combined with CD20 monoclonal antibody (mAb), is still the standard therapy for previously untreated IGHV-M CLL patients without TP53 aberrations (Parikh 2018). A combination of chemotherapy agents fludarabine and cyclophosphamide and anti-CD20 mAb rituximab (FCR) is used for young patients in good physical condition (Hallek et al. 2010). Another commonly used CIT regimen is a combination of bendamustine and rituximab (BR) particularly for patients over 65 years of age (Fischer et al. 2012). For older adults with significant comorbidities, the current standard treatment regimen is a combination of chemotherapy agent chlorambucil and anti-CD20 mAb obinutuzumab (Goede et al. 2015). For previously untreated CLL patients with the 17p13 deletion or TP53

mutation, ibrutinib, a Bruton´s tyrosine kinase (BTK) inhibitor, is the current standard treatment (Ahn et al. 2018; O'Brien et al. 2018).

For relapsed or CIT refractory patients, novel targeted agents are the most preferable option. The alternative regimens are ibrutinib, BCL2 inhibitor venetoclax, particularly in combination with anti-CD20 mAb, or PI3Kδ inhibitor idelalisib in combination with rituximab (Furman et al. 2014; Kater et al. 2019; O'Brien et al. 2018). For younger patients in good physical condition who carry TP53 abnormalities, allogeneic HSCT is also an option to consider (Dreger et al. 2018).

The rate of infections has not been significantly lower with novel regimens compared to CIT (Woyach et al. 2018). In the randomized studies, upper respiratory infections and pneumonia have been common infectious complications (Maschmeyer et al. 2019; Woyach et al. 2018).

2.3 Indoleamine 2,3-dioxygenase (IDO)

2.3.1 Background

The essential amino acid L-tryptophan (trp) is a precursor of serotonin, melatonin, and vitamin B3 (niacin). Trp is also required for protein synthesis (Le Floc'h et al. 2011; Stavrum et al. 2013). It is metabolized into kynurenine (kyn) through the kyn pathway leading to trp degradation (Figure 1). In this pathway, the initial rate-limiting step is catalyzed by IDO (Mellor & Munn 1999). IDO2 is structurally similar to IDO and catabolizes also tryptophan. IDO and IDO2 are encoded by genes IDO1 and IDO2, respectively (Ball et al. 2007; Ball et al. 2009; Munn & Mellor 2016). IDO1 is located adjacent to IDO2 in the short arm of chromosome 8 (Ball HJ 2009; Najfeld et al. 1993). IDO1 expression is regulated by interferons (IFNs) and enhanced by other inflammatory agents, such as bacterial lipopolysaccharide, interleukins (ILs), and tumor necrosis factor alpha (TNF-α) (Hissong et al. 1995; Robinson et al. 2003). IFNs induce immune regulatory responses mediated by IDO (Mellor & Munn 1999). IDO is expressed at least in macrophages, epithelial cells, DCs, and fibroblasts. In contrast, lymphoid cells rarely express IDO (Kahler & Mellor 2009). IDO2 expression is found particularly in the kidney (Ball et al. 2007). The biological role of IDO2 is not clear, but it may also be active in immunomodulation (Ball et al. 2009).

IDO suppresses T cell responses via the kyn and downstream metabolites (Mellor & Munn 1999). Trp depletion also arrests lymphocyte cell cycle progression and causes naive T cells to differentiate into regulatory T-cells (Treg) (Fallarino et al. 2006; Munn et al. 1999). FoxP3+ Treg cells are activated, increasing tolerance to inflammatory signals. IDO activity and expression are elevated in many chronic inflammatory diseases, infections, autoimmune diseases, and tumors (Mellor et al. 2017). Overexpression of IDO in tumors occurs in tumor cells or associated cells like DCs, macrophages, or endothelial cells. Elevated IDO expression mediates tolerogenic responses to tumor cells. Activation of suppressive Treg cells by IDO expressing antigen-presenting cells upregulates programmed cell death protein 1 (PD-1) expression and suppresses immune response against apoptotic tumor cells, thereby promoting tumor growth (Munn & Mellor 2016).

Overexpression of IDO has been found in many tumors including colon cancer, lung cancer, melanoma, and breast cancer (Brandacher et al. 2006; Curti et al. 2009; Isla Larrain et al. 2014; Schafer et al. 2016). In cancer immunotherapy, IDO may present a feasible target. IDO inhibitor drugs could enhance the immune responses triggered by other agents. Clinical trials in which IDO inhibitors are combined with chemotherapy, checkpoint inhibitors, or vaccines are ongoing (Munn & Mellor 2016).

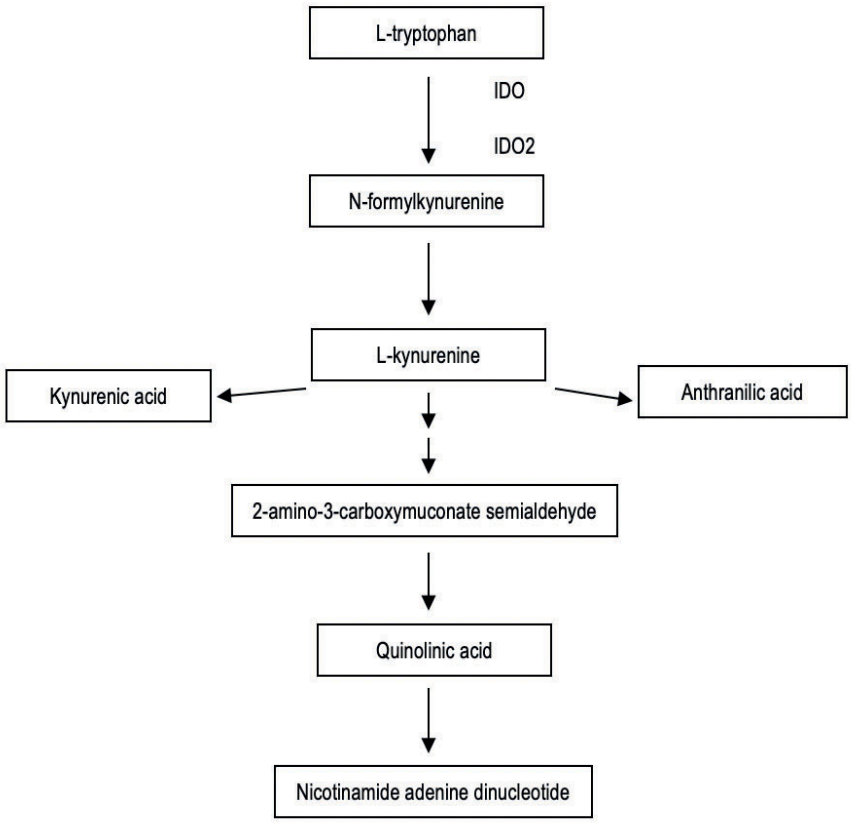

Figure 1. The kynurenine pathway of tryptophan metabolism (modified from Curti et al. 2009).

2.3.2 IDO in hematological malignancies

A few studies are available concerning IDO activity and expression in hematological malignancies. In a study with acute myeloid leukemia (AML) patients, the kyn/trp ratio was elevated, suggesting a raised IDO activity in AML compared to healthy controls (Corm et al. 2009). In another study, a significant portion of AML cells expressed IDO, whereas IDO was not detected in normal hematopoietic bone marrow cells. IDO expressed in AML cells was also found to be active, leading to depletion of trp (Curti et al. 2007). Furthermore, the proportion of FoxP3$^+$ Treg cells was significantly increased in IDO expressing AML cells at diagnosis by tryptophan

catabolism compared to healthy controls or AML cells that did not express IDO. In a mouse model, the formation of Tregs was blocked by IDO inhibitor 1-methyl tryptophan (Curti et al. 2007). Nimesulide, cyclooxygenase (COX)-2 inhibitor, has also been shown to inhibit IDO by depleting trp and abrogating kyn release in AML cells. IDO1 gene expression was also reduced in these cells. Furthermore, number of FoxP3+ Treg cells was diminished after exposure to nimesulide (Iachininoto et al. 2013). High IDO1 expression has also been found to impact prognosis of AML. In a study with 286 AML patients, high IDO1 expression correlated with a lower complete remission rate, and shorter overall and relapse-free survival (Chamuleau et al. 2008).

Increased IDO activity has been detected also in patients with multiple myeloma. In a study with 25 myeloma patients, 75% of patients showed increased kyn concentrations. Kyn was significantly increased particularly in patients with a more advanced stage of the disease. IDO activity also correlated with the expansion of functional Treg cells (Bonanno et al. 2012). In a recent study, IL-32 secreted by myeloma cells promoted IDO production in macrophages (Yan H et al. 2019).

In Hodgkin's lymphoma, high IDO expression has been shown in macrophages, DCs, and endothelial cells primarily in the mixed cellular type of the disease, but not in Hodgkin Reed Stenberg cells or lymphocytes. High IDO expression has also been found to correlate with significantly shortened survival (Choe et al. 2014).

2.4 Pneumococcal vaccines

2.4.1 Polysaccharide vaccine

The whole cell vaccine was the first pneumococcal vaccine studied in a clinical trial conducted in South African gold miners in 1911 (Austrian 1977). After the serotype specificity of pneumococcal infection was discovered, the serotype-specific pneumococcal polysaccharide vaccines (PPVs) replaced the whole cell vaccine (Geno et al. 2015). 14-valent PPV was introduced in 1977 and it was expanded to 23-valent (PPV23) in 1983 (Grabenstein & Klugman 2012). PPV23 is the only currently available polysaccharide (PS) vaccine.

PPV23 contains polysaccharides of 23 different serotypes. As polysaccharide antigens are T-independent, they exhibit poor immunogenic efficacy in young

children (Timens et al. 1989). PPV23 causes depletion of the peripheral memory B-cell population. Hence, antibody concentrations after subsequent doses of PPV23 are similar to or even lower than those seen after the primary dose. This phenomenon is called hyporesponsiveness (Clutterbuck et al. 2012). PPV23 has limited efficacy against IPD also in adults (Andrews et al. 2012; Huss et al. 2009).

2.4.2 Conjugate vaccine

Pneumococcal conjugate vaccines (PCVs) are immunogenic also in young infants and children (Black et al. 2000). Conjugation of a pneumococcal polysaccharide to a carrier protein enhances the immunogenicity of the polysaccharide. Conjugation transforms the T-cell independent polysaccharide vaccine to a T-dependent conjugate vaccine. PCVs' capability of creating immunological memory induces responsiveness to subsequent doses of vaccine. Antibodies generated by PCVs have higher avidity with better functional capacity (Geno et al. 2015; Pichichero 2013). Furthermore, PCVs reduce nasopharyngeal carriage, which is considered critical for the formation of herd immunity (Rashid et al. 2012). The serotypes included in PCVs have been selected on the basis of serotypes causing IPD in children (Rodgers & Klugman 2011). PCV7 was licensed in the US in 2000. Since then, PCV10 and PCV13 have been developed. PCV7 contained capsular PSs of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Serotypes 1, 5, and 7F were added to PCV10 and the currently used PCV13 also includes serotypes 3, 6A, and 19A (Geno et al. 2015).

Four carrier proteins have been used in pneumococcal conjugate vaccines. In PCV10 each serotype is conjugated to tetanus toxoid, diphteria toxoid, or a recombinant *Haemophilus influenzae* protein D. All serotypes of PCV7 and PCV13 are conjugated to genetically modified cross-reacting material of diphteria toxin (CRM_{197}) (Geno et al. 2015; Pichichero 2013).

2.4.3 Vaccination studies in hematological malignancies

The majority of pneumococcal vaccination studies in hematological malignancies have been performed in HSCT recipients. A randomized study of EBMT compared early administration of PCV with late vaccination after allogeneic HSCT. In the early vaccination group, a course of three doses of PCV7 given 1 month apart was initiated 3 months and in the late group 9 months after transplantation. The antibody response rate seen in the early vaccination group one month after the last PCV7 dose

was similar to the rate in the late group, indicating the benefits brought by earlier protection against IPD in these high-risk patients. However, at 1 year after HSCT, antibody concentrations in the early group declined significantly compared to the late group. Furthermore, after a single dose of PPV23 given at 12 and 18 months in the early and in the late group, respectively, 41% of the patients who were non-responders to PCV7 achieved a response (Cordonnier et al. 2009). PPV23 also expanded the serotype coverage for the antigens 1 and 5, which are not included in PCV7 (Cordonnier et al. 2010). Antibody persistence among long-term survivors in the same study population was also assessed 10 years after the vaccination program. In 40% of the patients, antibody concentrations remained at a protective level against IPD (Cordonnier et al. 2015). In a study with PCV13, pediatric and adult allogeneic HSCT recipients received three doses of PCV13 3-6 months after transplantation. A fourth dose of PCV13 was administered 6 months later and a dose of PPV23 1 month from the last dose of PCV13. After the third dose, antibody concentrations increased significantly for all PCV13 serotypes from the baseline. Before the fourth dose of PCV13, antibody concentrations declined but significantly increased again after the fourth dose. After the dose of PPV23, there were no changes in antibody concentrations (Cordonnier et al. 2015).

Since the beginning of the 21st century, only a few studies have been carried out on pneumococcal vaccines in patients with CLL. Most studies with PPV23 have shown no significant antibody responses (Hartkamp et al. 2001; Sinisalo et al. 2001). In addition, the administration of 3 doses of granulocyte-macrophage-colony-stimulating factor (GM-CSF) before or after the PPV23 did not improve antibody response (Safdar et al. 2008). Furthermore, ranitidine treatment inhibiting the histamine effect, thereby probably enhancing immunoglobulin production, did not improve antibody response to PPV23 (van der Velden et al. 2007). PCVs, in contrast, have shown efficacy in inducing antibody response in CLL patients. In a study with PCV7, almost 40% of CLL patients achieved a significant antibody response to at least six PCV7 antigens when the vaccine was administered at an early stage of the disease. Compared to immunocompetent controls, antibody concentrations were significantly lower in CLL patients after vaccination (Sinisalo et al. 2007). To date, two studies have been carried out on PCV13. In a study of 24 untreated CLL patients and 15 controls without previous PPV23 vaccination, 58% of the patients and all controls achieved at least a two-fold increase in antibody titers from the baseline with one dose of PCV13. The proportion of plasmablasts, suggesting early response to vaccination and stimulation of the immune system, was significantly higher than the baseline in CLL patients after vaccination while remaining significantly lower

than in controls (Pasiarski et al. 2014). In a recent randomized study, PCV13 was compared to PPV23 in untreated CLL patients. Functional antibody titers measured by opsonophagocytic assay (OPA) were significantly higher for 11 out of the 13 serotypes with PCV13 at one month after vaccination and for 6 out of the 13 serotypes at six months after vaccination compared to PPV23. All antibody responses for PPV23 were inferior to those generated by PCV13 (Svensson et al. 2018). Serotype-specific antibody responses are available from two studies (Table 1).

In patients with multiple myeloma, the immunogenicity of PPV23 is also poor. Only 33% of patients achieved a response to PPV23 when administrated before autologous HSCT at the time of peripheral stem cell harvest (Hinge et al. 2012). Instead, in a study with 20 patients with smoldering multiple myeloma, 60% of the patients achieved a significant antibody response for PCV7 serotypes measured by enzyme-linked immunosorbent assay (ELISA) after one dose of PCV13 at one month after vaccination. A significant response was achieved in 40% of the patients measured by OPA, defined as at least a 4-fold increase from the baseline. At 6 months after vaccination, 35% of the patients fulfilled the same response criteria by ELISA and 30% by OPA. At 12 months, these percentages were only 25 (ELISA) and 10% (OPA) (Bahuaud et al. 2017).

2.4.4 Vaccination guidelines

In the guidelines in place in the United States, PPV23 is recommended no less than 8 weeks after PCV13 to adults 19 years of age and older with immunocompromising conditions. A second dose of PPV23 is recommended 5 years after the first dose (Bennett et al. 2012). Furthermore, PPV23 is recommended for all adults 65 years of age and older one year after a dose of PCV13, or later (Kobayashi et al. 2015). In the guidelines of the European Conference on Infectious in Leukaemia (ECIL) group for patients with hematological malignancy, PCV13 followed by PPV23 no less than 8 weeks later is recommended to patients with multiple myeloma, lymphoma, and CLL before treatment. Patients with AML and myelodysplastic syndromes (MDS) are recommended to vaccinate 3-6 months after chemotherapy (Mikulska et al. 2019).

Table 1. Response rates in studies with pneumococcal conjugate vaccine in patients with CLL.

Serotype	Vaccine Method	Sinisalo et al. 2007 PCV7 ELISA	Svensson et al. 2018 PCV13 OPA
		Response rate (%)[a]	%[b]
4		35	61
6B		43	59
9V		20	52
14		39	78
18C		43	79
19F		47	49
23F		47	48

[a]Defined as an at least 2-fold increase and a post-vaccination concentration of at least 0.35 µg/ml
[b]OPA-titer ≥ LLOQ (lower limit of quantification)

3 AIMS OF THE STUDY

The main objectives of this study were to gain better understanding of the characteristics of IPD in patients with hematological malignancies, assess efficacy of pneumococcal vaccines especially in patients with CLL, and discover possible factors contributing to the vaccine responses in CLL patients.

The specific aims were:

1. To evaluate IDO activity and IDO1 and IDO2 gene expression in CLL.

2. To determine the incidence of IPD, serotype distribution, and serotype coverage of pneumococcal conjugate and polysaccharide vaccines in patients with specific hematological malignancies.

3. To assess antibody persistence in patients with CLL five years after the administration of PCV7.

4. To evaluate the efficacy of the PPV23 booster dose given five years after PCV7 in patients with CLL.

4 MATERIALS AND METHODS

4.1 Subjects and controls

4.1.1 Study I

For measurements of IDO activity, the study population included 49 patients with CLL and 24 age- and sex-matched controls from Tampere and Turku University Hospitals who had participated in an earlier vaccine response study with PCV7. A majority of the patients had an early stage disease. According to the Binet classification, 39 patients had stage A disease and 9 patients had stage B disease while 1 patient had stage C disease. Eleven patients had received chemotherapy (Sinisalo et al. 2007). The study population for IDO1 and IDO2 gene expression comprised 10 CLL patients and 7 controls. All 10 untreated CLL patients had stage A disease according to the Binet classification.

4.1.2 Study II

In Finland, clinical microbiology laboratories are required to report bacterial isolations from blood and cerebrospinal fluid (CSF), including *S.pneumoniae*, to the National Infectious Disease Register (NIDR), a population-based laboratory surveillance system. A case of IPD was defined as isolation of *S.pneumoniae* from blood and/or CSF during 1995-2002. To find data concerning diagnosis of specific hematological malignancy (Hodgkin´s lymphoma, non-Hodgkin´s lymphoma, myeloma, or leukemia), national IPD surveillance data were linked to the Finnish Cancer Registry database (Brenner & Hakulinen 2005; Klemets et al. 2008). Additionally, ICD (9th and 10th Revision) codes of hematological malignancy for the IPD episode were collected from the National Hospital Discharge Register (HILMO). The diagnoses of hematological malignancy within one year prior to the first episode of IPD were only analyzed to maintain the cumulative incidence of hematological malignancies constant. Of the total of 4611 IPD cases identified, only

the first episodes of disease (n=4357) were included in the analysis (Klemets et al. 2008). A hematological malignancy (Hodgkin's or non-Hodgkin's lymphoma, myeloma, or leukemia) had been diagnosed in 56 (1.3%) cases within one year prior to the IPD episode. Of these 56 *S.pneumoniae* isolates from patients with hematological malignancy and IPD, the strain was available for serotyping in 47 cases (84%). The median age of these patients was 64 years (range 9 months-81 years) at the time of IPD and 68% (n=32) of the isolates were from males. The isolation of bacteria was performed in 45 cases from blood (96%) and from CSF in 2 cases (4.3%). Only three patients had a specific CLL diagnosis.

4.1.3 Studies III and IV

The study population comprised 24 CLL patients (12 males and 12 females), with a median age of 64 years (range 47-86 years) from Tampere and Turku University Hospitals (Table 2). The control population consisted of eight subjects (median age 67 years, range 57-82 years, four males and four females) without any known immunological defects or hematological diseases from Tampere University Hospital. The patients and controls had participated in an earlier pneumococcal conjugate vaccine response study with PCV7 (Sinisalo et al. 2007). In study III, the disease status according to the Binet classification was A in 16, B in 2, and C in 6 patients. A total of 16 patients had never been treated for CLL. Seven patients had suffered from severe infections (requiring intravenous antibiotics or hospitalization) and six patients from mild to moderate infections (treated with oral antibiotics) during the five years since PCV7 vaccination. Only one of these infections was pneumococcal infection, i.e. pneumococcal septicemia. Hypogammaglobulinemia (S-IgG <6.77 g/l) was detected in 11 patients.

In study IV, samples taken after PPV23 administration were not available in the case of four patients. Hence, the final study population in study IV comprised 20 CLL patients and 8 controls. According to the Binet classification, the disease status for these patients was A in 12, B in 2, and C in 6 patients. Thirteen patients had never been treated for CLL. Six patients had received CIT or chemotherapy, one patient CD52 monoclonal antibody (alemtuzumab) after chemotherapy, and one patient had received allogeneic HSCT. Hypogammaglobulinemia as defined above was detected in nine (45%) patients. Of these 20 patients, five had suffered from severe infections and five from mild to moderate infections during the 5 years since PCV7 vaccination. Only one of these infections was invasive pneumococcal disease.

Table 2. Clinical and laboratory characteristics of patients with CLL in studies III and IV.

Character	Patients with CLL (n=24)
Sex M/F	12/12
Age (years)	64 (47-86)
Binet A/B/C	16/2/6
Past CLL therapy	8 (33%)
Lymphocyte count, $x10^9$/l (1.2-3.5)	24.3 (0.9-140.0)
Platelet count, $x10^9$/l (150-360)	141 (38-372)
Hemoglobin, g/l (117-167)	135 (81-153)
Neutrophil count, $x10^9$/l (1.6-6.2)	4.0 (0.6-12.1)
IgG, g/l (6.77-15)	7.4 (3.2-12.5)
IgM, g/l (0.36-2.84)	0.3 (0.1-5.4)
IgA, g/l (0.52-4.84)	0.7 (0.2-4.5)

The values are expressed as medians and ranges

4.2 Vaccination

The vaccine used in study IV was 23-valent pneumococcal polysaccharide vaccine (Pneumovax®), which contains capsular polysaccharides of pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. A dose of 0.5 ml contains 25 μg of each PS type and phenol as adjuvant (Robbins et al. 1983). It also contains significant amount of cell wall polysaccharide (Sorensen & Henrichsen 1984). Patients and controls received one intramuscular deltoid injection of Pneumovax® five years after PCV7.

4.3 Samples and processing

In study I, venous blood samples had been taken before PCV7 administration and stored at -20°C (Sinisalo et al. 2007).

In studies III and IV, venous blood samples for antibody analyses were taken before PPV23 administration (five years after the administration of PCV7) and one month after PPV23. Serum was separated by centrifugation and stored at -20°C. In addition, venous blood samples were drawn from the CLL patients at the time of PPV23 administration for the analysis of total blood count and immunoglobulins.

4.4 Methods

4.4.1 Determination of IDO activity and expression

Trp (mmol/l) and kyn (μmol/l) concentrations in peripheral blood were measured at the Department of Microbiology and Immunology of Tampere University by reverse-phase high-performance liquid chromatography (HPLC). Trp was separated with a Shimadzu liquid chromatograph LC-10AD VP (Shimadzu Co, Kyoto, Japan) using a 50-mm BDS Hypersil C18 5 μm column (Thermo Electron Co, Bellefonte, PA, USA). Monitoring was performed by fluorescence with a Shimadzu RF-10A XL detector at 266 nm excitation and 366 nm emission wavelengths. Kyn was separated with a Hewlett Packard 1100 liquid chromatograph (Palo Alto, CA, USA) using a Merck LiChroCart 55-4150 mm cartridge containing a Purospher STAR RP-18 3 μm column (Merck Co, Darmstadt, Germany) and analyzed by ultraviolet absorption at 360 nm wavelength with a Hewlett Packard G13144 detector. For IDO1 and IDO2 gene analysis, blood samples were directly subjected to leukocyte separation with Histopaque 1077 density gradient (Histopaque-1077, cat. no. 10771, Sigma-Aldrich, MO, USA). The peripheral blood mononuclear cell (PBMC) layer was collected, and the cells were suspended into 1 ml of RPMI-1640 medium (cat. no. R0883, Sigma-Aldrich, MO, USA). Immediately after the PBMC separation, traces of erythrocytes were lysed with 10 s of H_2O treatment and immediately recovered with 0.9% NaCl. Total ribonucleic acid (RNA) extraction was performed with the Qiagen RNEasy Midi kit (cat. no. 75144, Qiagen, CA, USA) according to the manufacturer´s instructions. The RNA (500ng) was converted into complementary deoxyribonucleic acid (cDNA) using the High Capacity cDNA Reverse Transcription Kit (cat. no. 4368814, Applied Biosystems, CA, USA) according to the manufacturer´s instructions. The isolated RNA was quantified spectrophotometrically (Nanodrop, Thermo Scientific, DE, USA) and tested for inhibition to achieve the correct RNA load for cDNA conversion. IDO activity was determined by calculating the kyn/trp (μmol/mmol) ratio by relating concentrations of kyn to trp.

Levels of IDO 1 and IDO2 gene transcripts were determined via the TaqMan real-time polymerase chain reaction (PCR). The levels of gene transcripts were analyzed with a separate single IDO1 gene expression assay (cat. no. Hs00158027_m1, Applied Biosystems, CA, USA) and an IDO2 gene expression assay (cat. no. Hs00401201_m1, Applied Biosystems, CA, USA) and TaqMan real-time PCR.

4.4.2 Calculation of incidence rates of IPD and serotyping

Incidence rates of IPD among patients with hematological malignancies including 95% confidence intervals were calculated using person-time of patients with a specific hematological malignancy in the Cancer Registry as a denominator. Data were analyzed by using SPSS Statistics 21.0 (Chicago, IL, USA).

S.pneumoniae isolates were serotyped at the National Institute for Health and Welfare (THL) reference laboratory (Klemets et al. 2008). Pneumococcal serotypes were grouped as PCV7, PCV10, PCV13, PPV23, and all other types.

4.4.3 Determination of pneumococcal antibodies

The concentrations of serum IgG antibody against pneumococcal capsular PSs were measured at the THL laboratory by a modification of the 22F inhibition enzyme immunoassay (EIA) method (Simell et al. 2008). In study III, antibodies to PCV7 serotypes 1, 3, 6B, 14, 19F, and 23F were determined. In addition, in study IV, together with PCV7 serotypes, PPV23 antigens 5 and 7F were determined, as an example of antigens which are not included in PCV7. The results are given as μg/ml calculated on the basis of the designated IgG values of the 89-SF reference serum (Simell et al. 2008). The determination limits of antibodies were 0.03 for serotype 4, 0.04 for 6B, 9V, 18C, and 23F, 0.06 for 19F, 0.1 for 7F and 14, and 0.15 μg/ml for serotype 5. In study III, an antibody concentration of 0.35 μg/ml was considered as the threshold for protection against IPD, as recommended by the World Health Organization WHO (Jodar et al. 2003). In study IV, a significant antibody response was defined as an at least two-fold increase from the baseline and a post-vaccination level of at least 0.35 μg/ml consistent with an earlier PCV7 study (Sinisalo et al. 2007).

4.4.4 Statistical analyses

In study I, Mann-Whitney *U*-test was applied in comparisons of kyn and trp concentrations and the kyn/trp ratio. Correlations were calculated by Spearman's rank correlation test. The gene expression results were analyzed with Relative Quantification (RQ) documents and the RQ Manager Software for automated data analysis (Applied Biosystems, CA, USA). The endogenous control for transcript was beta-actin (BACT, cat. no. Hs03023880_g1). The calculations were based on Δ

threshold cycles (Ct) values obtained by deducting the Ct value of the endogenous control from that of the given target gene. Then, the ΔΔCt values were calculated by deducting the ΔCt of the calibrator from the Ct value of both target genes. The RQ value, which indicates the fold change for both targets, was derived from the formula: $RQ = 2^{-\Delta\Delta Ct}$. A gene that was regulated more than twofold (≥2.0 or ≤0.5) was considered to be significantly regulated (Jylhävä et al. 2010).

In study III, a comparison of antibody concentrations five years after PCV7 administration between CLL patients and controls was performed with Fisher's exact test. In study IV, antibody concentrations and proportionate changes between groups were compared by independent-samples Mann-Whitney test and the significance of antibody responses within groups by 2-tailed Fisher's exact test.

4.4.5 Ethical considerations

In studies I, III, and IV, written informed consent was obtained from all patients and controls. Studies were approved by the ethical board of the Pirkanmaa Hospital District. In study II, the national registry data use was authorized by the Ministry of Social Affairs and Health, the Finnish Data Protection Authority, and the National Research and Development Center for Welfare and Health. Studies were conducted in accordance with the Declaration of Helsinki.

5 RESULTS

5.1 IDO activity and expression in patients with CLL

In CLL patients, the kyn/trp ratio was significantly higher than in controls, indicating increased IDO activity, but no statistically significant difference in kyn or trp concentrations was discovered. The correlation of the kyn/trp ratio was estimated against demographic and disease-associated parameters in CLL patients, including age, sex, Binet class, disease duration, immunoglobulin levels, hemoglobin level, blood cell counts (lymphocytes, neutrophils, thrombocytes), and C-reactive protein levels. A statistically significant association of kyn/trp ratio was detected with age ($r=0.367$, $p=0.010$) and disease duration ($r=0.290$, $p=0.044$). However, increased IDO activity did not affect the antibody response to PCV7 (Sinisalo et al. 2007). There was no difference in the kyn/trp ratio between responders and non-responders among CLL patients, and no significant correlations between the kyn/trp ratio and serotype-specific responses were found.

Instead of increased IDO activity in CLL patients, a significantly reduced expression of IDO1 and IDO2 genes was detected in PBMCs. Kyn and trp concentrations, the kyn/trp ratio, and IDO1 and IDO2 gene RNA expression in PBMCs in patients with CLL and controls are shown in Table 3.

Table 3. Kynurenine and tryptophan concentrations and their ratio (kyn/trp) and IDO1 and IDO2 gene RNA expression in peripheral blood mononuclear cells in patients with CLL and controls.

Character	CLL n=49	Controls n=24	p-value
Kyn/trp ratio, µmol/mmol	37 (31-48)	33 (29-38)	0.027
Kynurenine, µmol/l	3.6 (2.7-4.6)	3.1 (2.6-3.8)	0.260
Tryptophan, mmol/l	0.094 (0.083-0.107)	0.095 (0.083-0.112)	0.425
	n=10	n=7	RQ
IDO1, Av ΔCt	14.18	11.63	0.17
IDO2, Av ΔCt	15.36	12.26	0.12

Abbreviations: Av ΔCt, average (mean) Δ threshold cycles; RQ relative quantification
The results are expressed in medians (quartiles) unless otherwise stated

5.2 IPD in Finnish patients with hematological malignancies

The overall incidence rate of IPD among patients with hematological malignancies was 3.8 per 1000 person-years (Table 4). Patients with multiple myeloma had the highest rate of IPD (10.9 cases per 1000 person-years). The most common pneumococcal serotypes were 14 (n=8, 17%), 6B (n=5, 11%), 11 (n=3, 6.5%), 4 (n=3, 6.5%), and 19F (n=2, 4.3%). Of the serotyped isolates, 24 (51%), 27 (57%), and 30 (64%) were serotypes covered by PCV10, PCV13 and PPV23, respectively (Figure 2). A total of 15 out of the 47 (32%) isolates were not included in either PPV23 or PCV13. Serotype coverage varied in line with the type of hematological malignancy: In patients with myeloma, the serotypes included in PCV13 and PPV23 covered 12 (60%) and 14 (70%) of cases, respectively (Figure 3).

Table 4. Rates of invasive pneumococcal disease (IPD) in patients with hematological malignancy in Finland 1995-2002 within one year of hematological diagnosis.

Malignancy	Cases of specified malignancy (person-years)[a]	IPD cases with specified malignancy	Incidence rate/1000 person years (95% CI)
Myeloma	2393	26	11 (7.4-16)
Any leukemia	4201	17	4.1 (2.5-6.5)
NHL	7131	12	1.7 (1.0-3.0)
HL	1080	1	0.9 (0.1-6.6)
Total	14805	56	3.8 (2.9-4.9)

Abbreviations: HL Hodgkin's lymphoma; NHL Non-Hodgkin's lymphoma
[a]From Finnish Cancer Registry

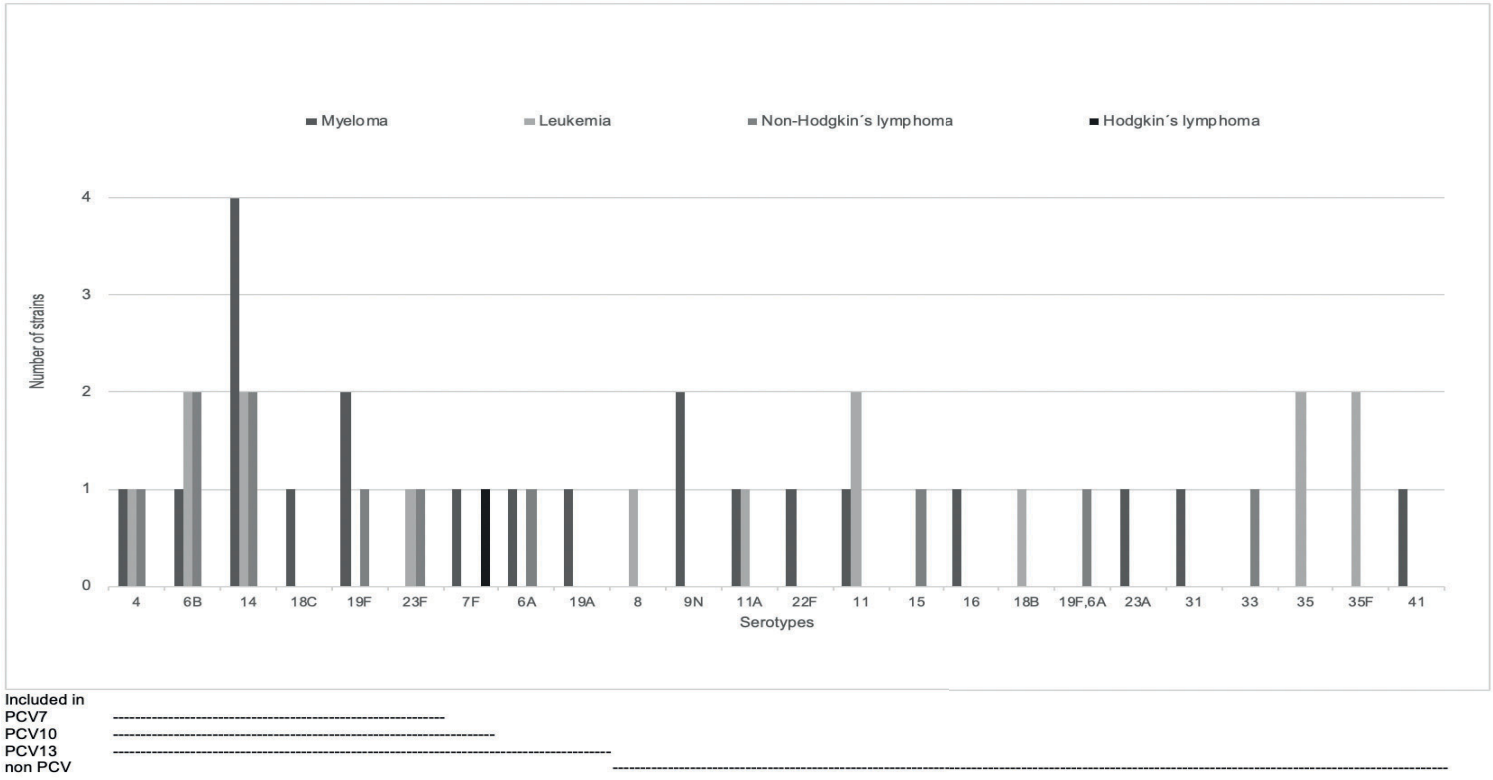

Figure 2. Distribution of serotypes causing IPD in patients with hematological malignancies in Finland during 1995-2002 within one year of diagnosis and serotype coverages of pneumococcal conjugate vaccines.

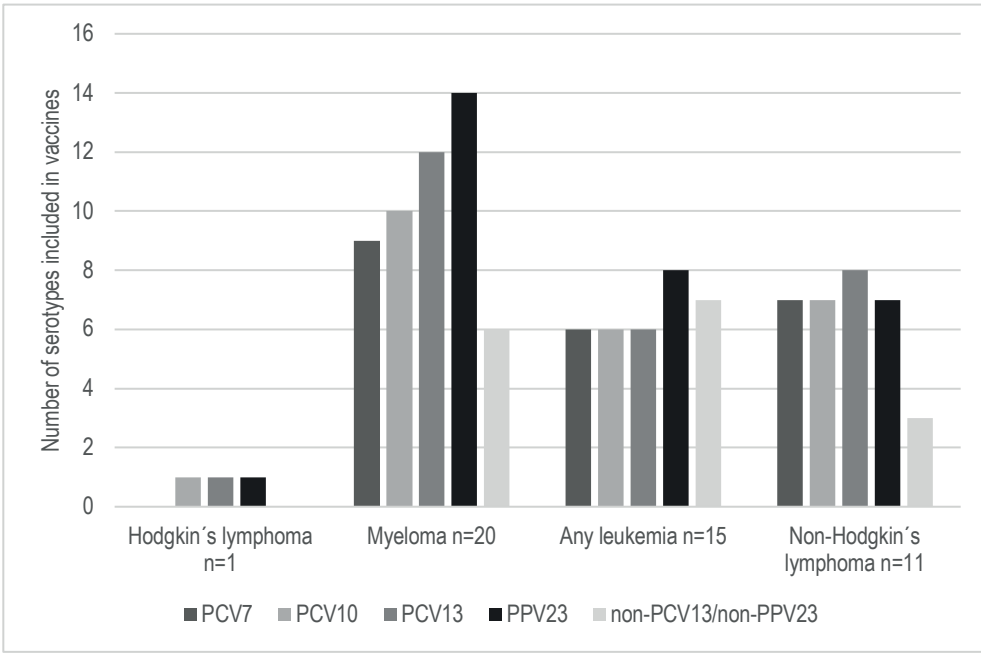

Figure 3. Number of IPD causing serotypes covered by pneumococcal vaccines in patients with hematological malignancies in Finland 1995-2002 within one year of diagnosis.

5.3 Antibody persistence after pneumococcal conjugate vaccine

In CLL patients, median antibody concentrations against pneumococcal serotypes 4, 6B, 18C, and 19F five years after PCV7 administration were approximately 50% lower than those measured four weeks after vaccination. Antibody concentrations against serotypes 9V and 23F were approximately 75% and 65% lower five years after vaccination, respectively. In contrast, the median concentration of the antibody against serotype 14 persisted at a similar level over the five-year period following the vaccination. In controls, antibody concentrations declined by more than 50% in each serotype group. The biggest decrease in controls was against serotype 9V, i.e. 95%. Between CLL patients and controls, no statistically significant differences were seen in antibody concentrations five years from vaccination (Table 5).

The baseline proportions of suggested protective antibody concentrations before a booster vaccination in patients with CLL ranged from 8 to 88%. The lowest baseline proportion was against serotype 4 and the highest against serotype 19F. In

controls, the same percentages were 0-100%, with no protective concentrations found against serotype 4. Protective antibody concentrations against serotype 18C were observed in all controls before vaccination. After five years of PCV7, the antibody concentrations in 29 to 71% of CLL patients, depending on serotype, remained at a level suggested to be protective against IPD (Figure 4). The lowest proportion was found against serotype 4 while the highest was against serotypes 18C and 19F. In controls, the corresponding percentages ranged from 50 to 87.5%, with the lowest proportion being against serotype 6B and the highest against serotype 18C.

Table 5. Antibody concentrations against pneumococcal antigens of 7-valent conjugate vaccine four weeks and five years after vaccination in patients with CLL and in controls.

Serotype	Post-PCV antibody level median (quartiles)		5 yrs post-PCV antibody level median (quartiles)		p-value[a]
	CLL	Control	CLL	Control	
4	0.30 (0.07-1.22)	1.87 (0.43-6.16)	0.15 (0.02-0.40)	0.52 (0.13-0.90)	0.116
6B	0.55 (0.20-1.82)	0.95 (0.23-14.7)	0.29 (0.09-0.94)	0.39 (0.06-1.24)	1.000
9V	1.40 (0.25-7.12	18.0 (0.34-63.2)	0.33 (0.15-2.33)	0.97 (0.17-5.06)	0.685
14	0.72 (0.31-2.66)	14.7 (1.01-20.5)	0.73 (0.20-4.01)	2.71 (0.45-5.83)	0.676
18C	1.55 (0.81-5.99)	9.76 (4.65-81.1)	0.73 (0.23-3.06)	1.42 (1.00-2.47)	0.642
19F	2.01 (0.61-9.31)	3.26 (1.20-51.1)	1.07 (0.26-2.88)	0.69 (0.31-1.56)	1.000
23F	1.53 (0.66-13.4)	5.11 (0.98-23.0)	0.51 (0.17-1.56)	1.17 (0.11-2.23)	1.000

[a]Fisher´s exact test
[a]Between CLL patients and controls five years from vaccination

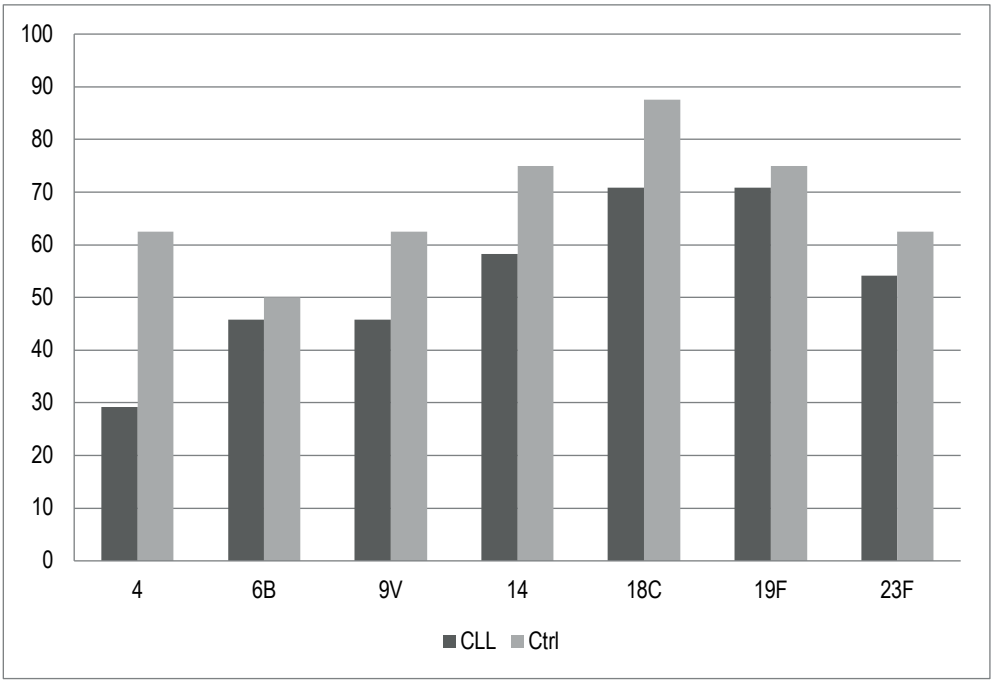

Figure 4. The proportions of antibody concentrations suggestive of protection (≥0.35 µg/ml) against pneumococcal antigens of 7-valent pneumococcal conjugate vaccine in patients with CLL and in controls (ctrl) five years after vaccine administration.

5.4 Antibody response of pneumococcal polysaccharide vaccine given five years after conjugate vaccine

Only 10 to 15% of CLL patients achieved a significant response to PCV7 antigens defined as an at least two-fold increase and a post-vaccination concentration of at least 0.35 µg/ml (Table 6). The responders were the same four patients, depending on the serotype. In controls, 75-88% achieved a significant response to PCV7 antigens 4, 6B, 9V, 14, 18C, and 19F. In contrast, the corresponding rate for serotype 23F was only 50%. For PPV23 antigens 5 and 7, the corresponding percentages of responders in CLL patients were 20% and 15%, respectively. All controls achieved a significant response to PPV23 antigens. Antibody concentrations after PPV23 were significantly higher in controls for four PCV7 antigens (4, 9V, 14, and 18C) and for both PPV23 antigens (Table 7). In contrast, no difference was observed in

antibody concentrations for PCV7 antigens 6B, 19F, and 23F. No vaccine-related adverse events were reported.

Table 6. Significant response rates and post-vaccination antibody concentrations suggestive of protection (≥0.35 µg/ml) to the seven serotypes included in PCV7 (bold) and to the two serotypes included in PPV23 in patients with CLL and controls.

	Response rate[a]			Post-vaccination concentration ≥0.35 µg/ml	
	CLL	Controls		CLL	Controls
Serotype	n=20 (%)	n=8 (%)	p-value[b]	n=20 (%)	n=8 (%
4	**2 (10)**	**7 (88)**	**<0.001**	**7 (35)**	**8 (100)**
5	4 (20)	8 (100)	<0.001	6 (30)	8 (100)
6B	**2 (10)**	**6 (75)**	**0.002**	**10 (50)**	**7 (88)**
7F	3 (15)	6 (75)	<0.001	14 (70)	8 (100)
9V	**3 (15)**	**7 (88)**	**0.001**	**13 (65)**	**8 (100)**
14	**2 (10)**	**7 (88)**	**<0.001**	**12 (60)**	**8 (100)**
18C	**2 (10)**	**6 (75)**	**0.002**	**15 (75)**	**8 (100)**
19F	**3 (15)**	**7 (88)**	**0.001**	**15 (75)**	**8 (100)**
23F	**2 (10)**	**4 (50)**	**0.038**	**13 (65)**	**8 (100)**

[a]Defined as an at least 2-fold increase and a post-vaccination concentration of at least 0.35 µg/ml
[b]Between significant response rates in patients with CLL and controls
[b]Fisher´s exact test

Table 7. Pre- and post-vaccination (PPV23) antibody concentrations to seven serotypes included in the 7-valent pneumococcal conjugate vaccine (bold) and two serotypes included in the 23-valent pneumococcal polysaccharide vaccine in CLL patients and in controls.

Serotype	Pre-vaccination antibody concentration GMC (µg/ml) (quartiles)		Post-vaccination antibody concentration GMC (µg/ml) (quartiles)		p-value[a]
	CLL (n=20)	Control (n=8)	CLL (n=20)	Control (n=8)	
4	**0.12 (0.02-0.40)**	**0.34 (0.13-0.90)**	**0.13 (0.02-0.67)**	**1.96 (1.36-2.82)**	**<0.001**
5	0.16 (0.08-0.44)	0.15 (0.08-0.25)	0.22 (0.08-0.64)	2.20 (0.53-5.57)	0.002
6B	**0.46 (0.11-1.69)**	**0.30 (0.06-1.24**	**0.56 (0.14-3.39)**	**2.28 (0.75-9.96)**	**0.063**
7F	0.38 (0.07-1.27)	0.56 (0.31-1.23)	0.55 (0.07-1.83)	6.74 (2.97-13.8)	<0.001
9V	**0.51 (0.23-2.33)**	**0.91 (0.17-5.06)**	**0.62 (0.32-2.29)**	**3.04 (0.58-12.1)**	**0.033**
14	**0.77 (0.20-4.14)**	**1.56 (0.45-5.83)**	**0.96 (0.17-6.20)**	**6.65 (3.00-24.0)**	**0.021**
18C	**0.93 (0.26-3.06)**	**1.55 (1.00-2.47)**	**1.06 (0.27-4.07)**	**6.81 (3.82-15.7)**	**0.010**
19F	**0.97 (0.30-2.88)**	**0.83 (0.31-1.56)**	**1.29 (0.33-4.20)**	**4.18 (1.88-6.05)**	**0.055**
23F	**0.53 (0.14-1.56)**	**0.82 (0.25-2.23**	**0.67 (0.13-4.41)**	**2.29 (2.07-3.32)**	**0.150**

Abbreviation: GMC geometric mean concentration

[a]Between post-vaccination concentrations in patients with CLL and controls

[a]Independent-samples Mann-Whitney U-test (statistical significance if $P \leq .05$)

6 DISCUSSION

This study focused on antibody persistence after pneumococcal conjugate vaccine and antibody response to the pneumococcal polysaccharide booster vaccine in CLL patients. Furthermore, the coverage of pneumococcal vaccines was analyzed. Another main goal was to estimate IDO activity and expression in CLL patients and to evaluate the possible impact of IDO on vaccine responses. Furthermore, this study assessed the incidence of IPD, serotype distribution, and coverage of pneumococcal vaccines in patients with hematological malignancies in Finland.

6.1 IDO and immune dysregulation in CLL

IDO activity was found to be increased in CLL patients based on their elevated kyn/trp ratio. This finding was consistent with other studies concerning IDO in hematological malignancies (Bonanno et al. 2012; Corm et al. 2009). IDO expression in PBMCs of CLL patients was reduced, suggesting that the observed IDO activity was derived from cells other than malignant B-lymphocytes. This finding has been confirmed with patients with Hodgkin's lymphoma, who express IDO in the microenvironment, but not in tumor cells or lymphocytes (Choe et el. 2014). As stated above, IDO expression is uncommon in lymphocytes (Kahler & Mellor 2009). Instead, AML blast cells have been shown to express IDO by themselves (Chamuleau et al. 2008; Curti et al. 2007).

Naive T-cells convert into Treg cells via IDO production and tryptophan catabolism (Fallarino et al. 2006). Treg cells play an important role in creating a tolerogenic microenvironment contributing to growth and survival of cancer cells (Munn & Mellor 2016; Ricciuti et al. 2018). An increased frequency of Treg cells has been discovered in AML, Hodgkin's lymphoma, non-Hodgkin's lymphoma, multiple myeloma, and myelodysplastic syndrome (Beyer et al. 2006; Kordasti et al. 2007; Marshall et al. 2004, Wang et al. 2005; Yang et al. 2006). Increased frequencies of CD4+CD25+ Treg cells have also been shown in patients with CLL, particularly in the advanced stage of the disease (Beyer et al. 2005). Although IDO expression via

increased IDO activity in CLL patients seems to originate from cells other than CLL cells themselves, as indicated by our data, it may play a role in the creation of an immunosuppressive microenvironment leading to tolerance to cancer cells.

Only very limited data is available concerning the role of Treg cells in vaccine responses. In a study with Gambian infants, Treg cells were found to suppress antibody response to measles vaccination but not to diphtheria-tetanus-pertussis vaccine (Ndure et al. 2017). In a study with hemodialysis patients, IDO levels were higher in patients with insufficient response to hepatitis B vaccine, compared to patients with adequate response (Eleftheriadis et al. 2011). In our data, we did not observe any impact of elevated IDO activity on vaccine antibody response.

6.2 Features of IPD in hematological malignancies

The risk of IPD in patients with hematological malignancies was 35 times higher than in the general population in Finland, where the average annual incidence of IPD was 0.11 cases per 1000 persons (Klemets et al. 2008). As established by earlier data, the highest rates were seen in patients with myeloma (Wong et al. 2010). The risk of IPD could have been expected to be even higher over a longer follow-up period. The pneumococcal serotype distribution among patients with hematological malignancy before the introduction of the infant conjugate vaccine program was different from serotypes causing IPD among the general population, as the most common serotypes among the general adult population were 4, 14, 3, 7F, and 23F and serotypes 6B, 14, 19A, 18C, and 7F among children (Klemets et al. 2008). Serotypes 14, 3, 7F, and 4 were also the most common ones among adults in 2001-2003 in North-Rhine Westphalia, Germany (Reinert et al. 2005). All the most common serotypes in our data were included in PPV23, but serotype 11 was not included in PCV13. Among the general population above the age of 18 in Finland, the serotype coverage of PCV13 and PPV23 was 1.3-fold higher than in the hematological patients in our study (Klemets et al. 2008). Among hematological patients and hematopoietic stem cell transplant recipients in France, the serotype coverage of PCV13 and PPV23 was 84% and 92%, respectively (Debbache et al. 2009). Hence, the proportion of those serotypes which were not covered in both PPV23 and PCV13 was relatively high in our study. The possible differences in serotype distribution and lower serotype coverage of pneumococcal vaccines may reduce the benefits from herd immunity. Patients with hematological malignancy would probably gain more protection against IPD from pneumococcal conjugate

vaccines with expanded serotype coverage as 15-valent pneumococcal conjugate vaccine (McFetridge et al. 2015).

6.3 Antibody persistence in patients with CLL

In patients with CLL, pneumococcal antibody concentrations declined during the five years following a single dose of PCV7 for six out of seven serotypes. In healthy controls, decline was seen for all serotypes. No statistically significant difference was seen between these two groups in median antibody concentrations. However, a trend toward lower antibody concentrations in CLL patients was seen compared to controls for all serotypes except for serotype 19F. The median antibody concentrations in CLL patients at five years after PCV7 administration, as compared with the baseline levels before vaccination, varied depending on the serotype. The post-vaccination antibody concentrations for serotypes 4 and 14 were 1.6- and 1.3 times higher than the baseline, respectively. In contrast, for serotypes 6B and 18C, the median antibody concentrations declined by almost 20% during the follow-up period. Antibody concentrations for the other three serotypes declined to baseline levels. However, in more than half of the CLL patients, the antibody concentrations remained at a level considered to be protective against IPD for four out of seven serotypes five years after PCV administration. Most of the patients had never been treated for CLL, which may have an impact on antibody persistence. The relatively low rates of hypogammaglobulinemia may also contribute to better antibody persistence.

There is no earlier data available concerning pneumococcal antibody persistence in patients with CLL. In the abovementioned data concerning serotype distribution of IPD in patients with hematological malignancy, serotypes 14 and 6B were the most common serotypes. The high persistence seen for serotype 14 offers support to the recommendation of early PCV administration to CLL patients. The decline in the serotype 6B antibody concentration to levels below the baseline may indicate a necessity of a booster vaccination.

6.4 Role of the polysaccharide booster vaccine

Earlier data concerning the antibody response of pneumococcal booster vaccine after conjugate vaccine was not available in CLL patients. It has been shown that

patients with Hodgkin's lymphoma and HSCT recipients benefit from a PCV primer before PPV23 (Chan et al. 1996; Cordonnier et al. 2009; Cordonnier et al. 2010). In our study, this booster effect was not observed in CLL patients. While PPV23 given one year after PCV13 improved PCV13 immunogenicity in adults with HIV, it had a negative effect on immunological memory induced by PCV13, causing hyporesponsiveness, thereby leading to the depletion of memory B-cells (Farmaki et al. 2018). Similar observations concerning depletion of immunological memory following PPV23 given after PCV have been reported (Papadatou & Spoulou 2016). Our study design was based on a long interval between vaccinations as this practice has been thought to decrease hyporesponsiveness, potentially providing better immunogenicity (Kobayashi et al. 2015). However, our data suggest that PCV is not capable of inducing properly functional memory B–cells in CLL patients. One probable reason for a poor antibody response with PPV23 after the PCV primer is abnormalities in both B- and T –cell functions even at an early stage of CLL, leading to the inadequate immunogenicity of conjugate vaccines (Dearden 2008; Morrison 2010; Pasiarski et al. 2014).

6.5 Limitations of the study and future perspectives

This study has some limitations. In study I, IDO expression was analyzed only in PBMCs but not in stromal cells to confirm IDO expression in a microenvironment. Furthermore, more far-reaching interpretation of data would have required analysis of functional Treg cells to confirm the formation of Treg cells as a result of increased IDO activation in CLL patients. These aspects should be verified in further studies as our knowledge of impact of IDO on tumor immunotolerance has broadened and research methods have evolved since our study period.

In study II, the pneumococcal vaccination status was not known, but based on a national survey in Finland the uptake of pneumococcal vaccines in the high-risk groups has been low (Ruutu et al. 2004). Furthermore, the study was performed in patients with hematological malignancies before the introduction of PCVs currently in use. As stated above, herd immunity may influence IPD serotype distribution to a lesser extent in patients with hematological malignancy. Hence, our results may reflect serotype distribution also in the era of PCVs. However, these data need to be confirmed in a patient population compiled after the introduction of PCVs.

In studies III and IV, the number of subjects remained quite small due to the relative rarity of the disease and the natural course of CLL. Since the study, PCV7

has been replaced with PCV13, but the data should be comparable due to the same carrier protein, CRM (Pichichero 2013). Furthermore, PCV13 has also been shown to generate suboptimal responses in CLL patients (Pasiarski et al. 2014). Still, more studies with the PCV13 primer are warranted in patients with CLL. Whether CLL patients would benefit from a PCV13 booster instead of PPV23 also needs to be studied.

7 SUMMARY AND CONCLUSIONS

Patients with hematological malignancies are at a high risk for IPD. Morbidity and mortality of IPD have remained significant in this group of patients even in the era of PCVs. Also, the uptake of pneumococcal vaccines is still inadequate within this patient group. CLL patients in particular have profound disturbances in immunoregulatory functions and the efficacy of pneumococcal vaccines has been limited. Due to the lack of comprehensive data, the optimal vaccination strategies are still unclear.

The IDO enzyme suppresses T cell functions and increased IDO activation and expression have been shown in malignant disease. IDO may contribute to the induction of tolerance to tumor cells and the occurrence of disturbances in immune functions. In CLL patients, the role of the IDO has not been previously studied.

In this study, the main objectives were to assess the incidence rate and serotype distribution of IPD and the serotype coverage of pneumococcal vaccines in patients with hematological malignancy. In CLL patients, we studied the persistence of PCV-induced antibody responses and the efficacy of the PPV23 booster vaccine. Furthermore, IDO activation and expression and their possible impact on vaccine responses in CLL patients were evaluated.

The main findings and conclusions in studies I to IV were:

1. IDO activity was increased in patients with CLL. However, IDO expression in malignant B-cells was reduced reflecting increased IDO activity arising from stromal cells. Influence of increased IDO activity on PCV7 antibody was not observed. Increased IDO activity may have a role in interactions between CLL cells and the microenvironment but these findings require further studies.
2. Before the use of PCVs, the risk of IPD in Finnish patients with hematological malignancies was clearly higher than that seen in the general population. Serotype distribution also seemed to differ from the distribution in the general population. Furthermore, serotype coverage of pneumococcal vaccines was lower in patients with hematological malignancies. These findings indicate that the predisposition to IPD in these

patients still remains high. To confirm these results, studies covering the PCV era are warranted.

3. After PCV7, the antibody concentrations remained at a level considered to be protective against IPD for four out of seven serotypes in more than 50% of the CLL patients for at least five years. There were no statistically significant differences in antibody concentrations between CLL patients and controls over this period, but a trend toward lower concentrations was seen in CLL patients for six serotypes.
4. The efficacy of a PPV23 dose given five years after PCV7 in CLL patients did not seem to be sufficient to induce an antibody response suggested to be protective against IPD. Future studies are still warranted to identify an optimal pneumococcal vaccination strategy in this high-risk patient population.

REFERENCES

Ahn, I. E., Farooqui, M. Z. H., Tian, X., Valdez, J., Sun, C., Soto, S., . . . Wiestner, A. (2018). Depth and durability of response to ibrutinib in CLL: 5-year follow-up of a phase 2 study. Blood, 131(21), 2357-2366.

Aittoniemi, J., Miettinen, A., Laine, S., Sinisalo, M., Laippala, P., Vilpo, L., & Vilpo, J. (1999). Opsonising immunoglobulins and mannan-binding lectin in chronic lymphocytic leukemia. Leukemia & Lymphoma, 34(3-4), 381-385.

Andrews, N. J., Waight, P. A., George, R. C., Slack, M. P., & Miller, E. (2012). Impact and effectiveness of 23-valent pneumococcal polysaccharide vaccine against invasive pneumococcal disease in the elderly in England and Wales. Vaccine, 30(48), 6802-6808.

Austrian, R. (1977). Prevention of pneumococcal infection by immunization with capsular polysaccharides of streptococcus pneumoniae: Current status of polyvalent vaccines. The Journal of Infectious Diseases, 136 Suppl, S38-42.

Backhaus, E., Berg, S., Andersson, R., Ockborn, G., Malmström, P., Dahl, M., . . . Trollfors, B. (2016). Epidemiology of invasive pneumococcal infections: Manifestations, incidence and case fatality rate correlated to age, gender and risk factors. BMC Infectious Diseases, 16, 367-016-1648-2.

Bahuaud, M., Bodilis, H., Malphettes, M., Maugard Landre, A., Matondo, C., Bouscary, D., . . . Fermand, J. P. (2017). Immunogenicity and persistence of the 13-valent pneumococcal conjugate vaccine (PCV13) in patients with untreated smoldering multiple myeloma (SMM): A pilot study. Heliyon, 3(11), e00441.

Ball, H. J., Sanchez-Perez, A., Weiser, S., Austin, C. J., Astelbauer, F., Miu, J., . . . Hunt, N. H. (2007). Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. Gene, 396(1), 203-213.

Ball, H. J., Yuasa, H. J., Austin, C. J., Weiser, S., & Hunt, N. H. (2009). Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. The International Journal of Biochemistry & Cell Biology, 41(3), 467-471.

Bennett, N. M., Whitney, C. G., Moore, M., Pilishvili, T., & Dooling, K. L. (2012). Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine for adults with immunocompromising conditions: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep., 61(40),816-819.

Beyer, M., Kochanek, M., Darabi, K., Popov, A., Jensen, M., Endl, E., . . . Schultze, J. L. (2005). Reduced frequencies and suppressive function of CD4+CD25hi regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. Blood, 106(6), 2018-2025.

Beyer, M., Kochanek, M., Giese, T., Endl, E., Weihrauch, M. R., Knolle, P. A., . . . Schultze, J. L. (2006). In vivo peripheral expansion of naive CD4+CD25high FoxP3+ regulatory T cells in patients with multiple myeloma. Blood, 107(10), 3940-3949.

Binet, J. L., Auquier, A., Dighiero, G., Chastang, C., Piguet, H., Goasguen, J., . . . Gremy, F. (1981). A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer, 48(1), 198-206.

Black, S., Shinefield, H., Fireman, B., Lewis, E., Ray, P., Hansen, J. R., . . . Edwards, K. (2000). Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. The Pediatric Infectious Disease Journal, 19(3), 187-195.

Bogaert, D., De Groot, R., & Hermans, P. W. (2004). Streptococcus pneumoniae colonisation: The key to pneumococcal disease. The Lancet.Infectious Diseases, 4(3), 144-154.

Bonanno, G., Mariotti, A., Procoli, A., Folgiero, V., Natale, D., De Rosa, L., . . . Rutella, S. (2012). Indoleamine 2,3-dioxygenase 1 (IDO1) activity correlates with immune system abnormalities in multiple myeloma. Journal of Translational Medicine, 10, 247-5876-10-247.

Brandacher, G., Perathoner, A., Ladurner, R., Schneeberger, S., Obrist, P., Winkler, C., . . . Amberger, A. (2006). Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: Effect on tumor-infiltrating T cells. Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 12(4), 1144-1151.

Breiman, RF., Butler, JC., & Nuorti, JP. (1997). Prevention of pneumococcal disease: Recommendations of the advisory committee on immunization practices (ACIP). MMWR Morb Mortal Wkly Rep., 46(RR-8), 1-24.

Brenner, H., & Hakulinen, T. (2005). Reduction in selective under-ascertainment bias in population-based estimates of cancer patient survival by age adjustment. European Journal of Cancer, 41(12), 1788-1793.

Briles, D. E., Crain, M. J., Gray, B. M., Forman, C., & Yother, J. (1992). Strong association between capsular type and virulence for mice among human isolates of streptococcus pneumoniae. Infection and Immunity, 60(1), 111-116.

Burger, J. A., Ghia, P., Rosenwald, A., & Caligaris-Cappio, F. (2009). The microenvironment in mature B-cell malignancies: A target for new treatment strategies. Blood, 114(16), 3367-3375.

Camara, J., Marimon, J. M., Cercenado, E., Larrosa, N., Quesada, M. D., Fontanals, D., . . . Ardanuy, C. (2017). Decrease of invasive pneumococcal disease (IPD) in adults after introduction of pneumococcal 13-valent conjugate vaccine in Spain. PloS One, 12(4), e0175224.

Chamuleau, M. E., van de Loosdrecht, A. A., Hess, C. J., Janssen, J. J., Zevenbergen, A., Delwel, R., . . . Ossenkoppele, G. J. (2008). High INDO (indoleamine 2,3-dioxygenase) mRNA level in blasts of acute myeloid leukemic patients predicts poor clinical outcome. Haematologica, 93(12), 1894-1898.

Chapman, K. E., Wilson, D., & Gorton, R. (2013). Invasive pneumococcal disease and socioeconomic deprivation: A population study from the North East of England. Journal of Public Health (Oxford, England), 35(4), 558-569.

Chan, C. Y., Molrine, D. C., George, S., Tarbell, N. J., Mauch, P., Diller, L., . . . Ambrosino, D. M. (1996). Pneumococcal conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine after treatment of Hodgkin's disease. The Journal of Infectious Diseases, 173(1), 256-258.

Chiorazzi, N., Rai, K. R., & Ferrarini, M. (2005). Chronic lymphocytic leukemia. N Engl J Med, 352(8), 804-815.

Choe, J. Y., Yun, J. Y., Jeon, Y. K., Kim, S. H., Park, G., Huh, J. R., . . . Kim, J. E. (2014). Indoleamine 2,3-dioxygenase (IDO) is frequently expressed in stromal cells of Hodgkin lymphoma and is associated with adverse clinical features: A retrospective cohort study. BMC Cancer, 14, 335-2407-14-335.

Clutterbuck, E. A., Lazarus, R., Yu, L. M., Bowman, J., Bateman, E. A., Diggle, L., . . . Pollard, A. J. (2012). Pneumococcal conjugate and plain polysaccharide vaccines have divergent effects on antigen-specific B cells. The Journal of Infectious Diseases, 205(9), 1408-1416.

Cohen, A. L., Harrison, L. H., Farley, M. M., Reingold, A. L., Hadler, J., Schaffner, W., . . . Active Bacterial Core Surveillance Team. (2010). Prevention of invasive pneumococcal disease among HIV-infected adults in the era of childhood pneumococcal immunization. AIDS, 24(14), 2253-2262.

Corcoran, M., Vickers, I., Mereckiene, J., Murchan, S., Cotter, S., Fitzgerald, M., . . . Humphreys, H. (2017). The epidemiology of invasive pneumococcal disease in older adults in the post-PCV era. Has there been a herd effect? Epidemiology and Infection, 145(11), 2390-2399.

Cordonnier, C., Labopin, M., Chesnel, V., Ribaud, P., Camara Rde, L., Martino, R., . . . Ljungman, P. (2010). Immune response to the 23-valent polysaccharide pneumococcal vaccine after the 7-valent conjugate vaccine in allogeneic stem cell transplant recipients: Results from the EBMT IDWP01 trial. Vaccine, 28(15), 2730-2734.

Cordonnier, C., Labopin, M., Chesnel, V., Ribaud, P., De La Camara, R., Martino, R., . . . Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. (2009). Randomized study of early versus late immunization with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. Clinical Infectious Diseases, 48(10), 1392-1401.

Cordonnier, C., Labopin, M., Robin, C., Ribaud, P., Cabanne, L., Chadelat, C., . . . Ljungman, P. (2015). Long-term persistence of the immune response to antipneumococcal vaccines after allo-SCT: 10-year follow-up of the EBMT-IDWP01 trial. Bone Marrow Transplantation, 50(7), 978-983.

Cordonnier, C., Ljungman, P., Juergens, C., Maertens, J., Selleslag, D., Sundaraiyer, V., . . . 3003 Study Group. (2015). Immunogenicity, safety, and tolerability of 13-valent pneumococcal conjugate vaccine followed by 23-valent pneumococcal polysaccharide vaccine in recipients of allogeneic hematopoietic stem cell transplant aged ≥2 years: An open-label study. Clinical Infectious Diseases, 61(3), 313-323.

Corm, S., Berthon, C., Imbenotte, M., Biggio, V., Lhermitte, M., Dupont, C., . . . Quesnel, B. (2009). Indoleamine 2,3-dioxygenase activity of acute myeloid leukemia cells can be measured from patients' sera by HPLC and is inducible by IFN-gamma. Leukemia Research, 33(3), 490-494.

Curti, A., Aluigi, M., Pandolfi, S., Ferri, E., Isidori, A., Salvestrini, V., . . . Lemoli, R. M. (2007). Acute myeloid leukemia cells constitutively express the immunoregulatory enzyme indoleamine 2,3-dioxygenase. Leukemia, 21(2), 353-355.

Curti, A., Pandolfi, S., Valzasina, B., Aluigi, M., Isidori, A., Ferri, E., . . . Lemoli, R. M. (2007). Modulation of tryptophan catabolism by human leukemic cells results in the conversion of CD25- into CD25+ T regulatory cells. Blood, 109(7), 2871-2877.

Curti, A., Trabanelli, S., Salvestrini, V., Baccarani, M., & Lemoli, R. M. (2009). The role of indoleamine 2,3-dioxygenase in the induction of immune tolerance: Focus on hematology. Blood, 113(11), 2394-2401.

Davey, F. R., Kurec, A. S., Tomar, R. H., & Smith, J. R. (1987). Serum immunoglobulins and lymphocyte subsets in chronic lymphocytic leukemia. American Journal of Clinical Pathology, 87(1), 60-65.

Dearden, C. (2008). Disease-specific complications of chronic lymphocytic leukemia. ASH Education Program Book, 2008(1), 450-456.

Debbache, K., Varon, E., Hicheri, Y., Legrand, P., Donay, J. L., Ribaud, P., & Cordonnier, C. (2009). The epidemiology of invasive streptococcus pneumoniae infections in onco-haematology and haematopoietic stem cell transplant patients in France. Are the serotypes covered by the available anti-pneumococcal vaccines? Clinical Microbiology and Infection, 15(9), 865-868.

Del Giudice, I., Rossi, D., Chiaretti, S., Marinelli, M., Tavolaro, S., Gabrielli, S., . . . Foa, R. (2012). NOTCH1 mutations in +12 chronic lymphocytic leukemia (CLL) confer an unfavorable prognosis, induce a distinctive transcriptional profiling and refine the intermediate prognosis of +12 CLL. Haematologica, 97(3), 437-441.

Dohner, H., Stilgenbauer, S., Benner, A., Leupolt, E., Krober, A., Bullinger, L., . . . Lichter, P. (2000). Genomic aberrations and survival in chronic lymphocytic leukemia. The New England Journal of Medicine, 343(26), 1910-1916.

Dreger, P., Ghia, P., Schetelig, J., van Gelder, M., Kimby, E., Michallet, M., . . . European Research Initiative on CLL (ERIC) and the European Society for Blood and Marrow Transplantation (EBMT). (2018). High-risk chronic lymphocytic leukemia in the era of pathway inhibitors: Integrating molecular and cellular therapies. Blood, 132(9), 892-902.

Drijkoningen, J. J., & Rohde, G. G. (2014). Pneumococcal infection in adults: Burden of disease. Clinical Microbiology and Infection, 20 Suppl 5, 45-51.

Eleftheriadis, T., Liakopoulos, V., Antoniadi, G., Stefanidis, I., & Galaktidou, G. (2011). Indoleamine 2,3-dioxygenase is increased in hemodialysis patients and affects immune response to hepatitis B vaccination. Vaccine, 29(12), 2242-2247.

Engelhard, D., Cordonnier, C., Shaw, P. J., Parkkali, T., Guenther, C., Martino, R., . . . Infectious Disease Working Party of the European Bone Marrow Transplantation (IDWP-EBMT). (2002). Early and late invasive pneumococcal infection following stem cell transplantation: A European bone marrow transplantation survey. British Journal of Haematology, 117(2), 444-450.

Fabbri, G., & Dalla-Favera, R. (2016). The molecular pathogenesis of chronic lymphocytic leukaemia. Nature Reviews.Cancer, 16(3), 145-162.

Fallarino, F., Grohmann, U., You, S., McGrath, B. C., Cavener, D. R., Vacca, C., . . . Puccetti, P. (2006). Tryptophan catabolism generates autoimmune-preventive regulatory T cells. Transplant Immunology, 17(1), 58-60.

Farmaki, P. F., Chini, M. C., Mangafas, N. M., Tzanoudaki, M. T., Piperi, C. P., Lazanas, M. Z., & Spoulou, V. S. (2018). Immunogenicity and immunological memory induced by the 13-valent pneumococcal conjugate followed by the 23-valent polysaccharide vaccine in HIV-infected adults. The Journal of Infectious Diseases, 218(1), 26-34.

Fischer, K., Cramer, P., Busch, R., Bottcher, S., Bahlo, J., Schubert, J., . . . Wendtner, C. M. (2012). Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: A multicenter phase II trial of the german chronic lymphocytic leukemia study group. Journal of Clinical Oncology, 30(26), 3209-3216.

Forconi, F., & Moss, P. (2015). Perturbation of the normal immune system in patients with CLL. Blood, 126(5), 573-581.

Francis, S., Karanth, M., Pratt, G., Starczynski, J., Hooper, L., Fegan, C., . . . Delgado, J. (2006). The effect of immunoglobulin VH gene mutation status and other prognostic factors on the incidence of major infections in patients with chronic lymphocytic leukemia. Cancer, 107(5), 1023-1033.

Furman, R. R., Sharman, J. P., Coutre, S. E., Cheson, B. D., Pagel, J. M., Hillmen, P., . . . O'Brien, S. M. (2014). Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. The New England Journal of Medicine, 370(11), 997-1007.

Geno, K. A., Gilbert, G. L., Song, J. Y., Skovsted, I. C., Klugman, K. P., Jones, C., . . . Nahm, M. H. (2015). Pneumococcal capsules and their types: Past, present, and future. Clinical Microbiology Reviews, 28(3), 871-899.

Goede, V., Fischer, K., Engelke, A., Schlag, R., Lepretre, S., Montero, L. F., . . . Hallek, M. (2015). Obinutuzumab as frontline treatment of chronic lymphocytic leukemia: Updated results of the CLL11 study. Leukemia, 29(7), 1602-1604.

Grabenstein, J. D., & Klugman, K. P. (2012). A century of pneumococcal vaccination research in humans. Clinical Microbiology and Infection, 18 Suppl 5, 15-24.

Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Dohner, H., . . . Kipps, T. J. (2018). iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. Blood, 131(25), 2745-2760.

Hallek, M., Fischer, K., Fingerle-Rowson, G., Fink, A. M., Busch, R., Mayer, J., . . . German Chronic Lymphocytic Leukaemia Study Group. (2010). Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: A randomised, open-label, phase 3 trial. Lancet, 376(9747), 1164-1174.

Hamblin, A. D., & Hamblin, T. J. (2008). The immunodeficiency of chronic lymphocytic leukaemia. British Medical Bulletin, 87, 49-62.

Hamblin, T. J., Davis, Z., Gardiner, A., Oscier, D. G., & Stevenson, F. K. (1999). Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood, 94(6), 1848-1854.

Hanquet, G., Krizova, P., Valentiner-Branth, P., Ladhani, S. N., Nuorti, J. P., Lepoutre, A., . . . SpIDnet/I-MOVE+ Pneumo Group. (2018). Effect of childhood pneumococcal conjugate vaccination on invasive disease in older adults of 10 European countries: Implications for adult vaccination. Thorax, doi:thoraxjnl-2018-211767 [pii]

Harboe, Z. B., Benfield, T. L., Valentiner-Branth, P., Hjuler, T., Lambertsen, L., Kaltoft, M., . . . Konradsen, H. B. (2010). Temporal trends in invasive pneumococcal disease and pneumococcal serotypes over 7 decades. Clinical Infectious Diseases, 50(3), 329-337.

Harboe, Z. B., Dalby, T., Weinberger, D. M., Benfield, T., Molbak, K., Slotved, H. C., . . . Valentiner-Branth, P. (2014). Impact of 13-valent pneumococcal conjugate vaccination in invasive pneumococcal disease incidence and mortality. Clinical Infectious Diseases, 59(8), 1066-1073.

Hartkamp, A., Mulder, A. H., Rijkers, G. T., van Velzen-Blad, H., & Biesma, D. H. (2001). Antibody responses to pneumococcal and haemophilus vaccinations in patients with B-cell chronic lymphocytic leukaemia. Vaccine, 19(13-14), 1671-1677.

Hashimoto, S., Dono, M., Wakai, M., Allen, S. L., Lichtman, S. M., Schulman, P., . . . Chiorazzi, N. (1995). Somatic diversification and selection of immunoglobulin heavy and light chain variable region genes in IgG+ CD5+ chronic lymphocytic leukemia B cells. The Journal of Experimental Medicine, 181(4), 1507-1517.

Herman, S. E., Gordon, A. L., Hertlein, E., Ramanunni, A., Zhang, X., Jaglowski, S., . . . Byrd, J. C. (2011). Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. Blood, 117(23), 6287-6296.

Hinge, M., Ingels, H. A., Slotved, H. C., & Molle, I. (2012). Serologic response to a 23-valent pneumococcal vaccine administered prior to autologous stem cell transplantation in patients with multiple myeloma. APMIS, 120(11), 935-940.

Hissong, B. D., Byrne, G. I., Padilla, M. L., & Carlin, J. M. (1995). Upregulation of interferon-induced indoleamine 2,3-dioxygenase in human macrophage cultures by lipopolysaccharide, muramyl tripeptide, and interleukin-1. Cellular Immunology, 160(2), 264-269.

Huss, A., Scott, P., Stuck, A. E., Trotter, C., & Egger, M. (2009). Efficacy of pneumococcal vaccination in adults: A meta-analysis. CMAJ, 180(1), 48-58.

Hyams, C., Camberlein, E., Cohen, J. M., Bax, K., & Brown, J. S. (2010). The streptococcus pneumoniae capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. Infection and Immunity, 78(2), 704-715.

Iachininoto, M. G., Nuzzolo, E. R., Bonanno, G., Mariotti, A., Procoli, A., Locatelli, F., . . . Rutella, S. (2013). Cyclooxygenase-2 (COX-2) inhibition constrains indoleamine 2,3-dioxygenase 1 (IDO1) activity in acute myeloid leukaemia cells. Molecules, 18(9), 10132-10145.

Ingels, H., Rasmussen, J., Andersen, P. H., Harboe, Z. B., Glismann, S., Konradsen, H., . . . Danish Pneumococcal Surveillance Collaboration Group 2009-2010. (2012). Impact of pneumococcal vaccination in Denmark during the first 3 years after PCV introduction in the childhood immunization programme. Vaccine, 30(26), 3944-3950.

Isla Larrain, M. T., Rabassa, M. E., Lacunza, E., Barbera, A., Creton, A., Segal-Eiras, A., & Croce, M. V. (2014). IDO is highly expressed in breast cancer and breast cancer-derived circulating microvesicles and associated to aggressive types of tumors by in silico analysis. Tumour Biology, 35(7), 6511-6519.

Itälä, M., Vainio, O., & Remes, K. (1996). Functional abnormalities in granulocytes predict susceptibility to bacterial infections in chronic lymphocytic leukaemia. European Journal of Haematology, 57(1), 46-53.

Jodar, L., Butler, J., Carlone, G., Dagan, R., Goldblatt, D., Käyhty, H., . . . Cherian, T. (2003). Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine, 21(23), 3265-3272.

Jylhävä, J., Eklund, C., Jylhä, M., Hervonen, A., & Hurme, M. (2010). Expression profiling of immune-associated genes in peripheral blood mononuclear cells reveals baseline differences in co-stimulatory signalling between nonagenarians and younger controls: The vitality 90+ study. Biogerontology, 11(6), 671-677.

Kadioglu, A., Weiser, J. N., Paton, J. C., & Andrew, P. W. (2008). The role of streptococcus pneumoniae virulence factors in host respiratory colonization and disease. Nature Reviews.Microbiology, 6(4), 288-301.

Kahler, D. J., & Mellor, A. L. (2009). T cell regulatory plasmacytoid dendritic cells expressing indoleamine 2,3 dioxygenase. Handbook of Experimental Pharmacology, (188):165-96. doi(188), 165-196.

Kater, A. P., Seymour, J. F., Hillmen, P., Eichhorst, B., Langerak, A. W., Owen, C., . . . Kipps, T. J. (2019). Fixed duration of venetoclax-rituximab in relapsed/refractory chronic lymphocytic leukemia eradicates minimal residual disease and prolongs survival: Post-treatment follow-up of the MURANO phase III study. Journal of Clinical Oncology, 37(4), 269-277.

Klemets, P., Lyytikäinen, O., Ruutu, P., Kaijalainen, T., Leinonen, M., Ollgren, J., & Nuorti, J. P. (2008). Trends and geographical variation in invasive pneumococcal infections in Finland. Scandinavian Journal of Infectious Diseases, 40(8), 621-628.

Klemets, P., Lyytikäinen, O., Ruutu, P., Ollgren, J., & Nuorti, J. P. (2008). Invasive pneumococcal infections among persons with and without underlying medical conditions: Implications for prevention strategies. BMC Infectious Diseases, 8, 96-2334-8-96.

Kobayashi M, Bennett NM, Gierke R, Almendares O, Moore MR, Whitney CG, Pilishvili T. (2015). Intervals between PCV13 and PPSV23 vaccines: Recommendations of the advisory committee on immunization practices (ACIP). MMWR Morb Mortal Wkly Rep., 34(64), 944-947.

Kordasti, S. Y., Ingram, W., Hayden, J., Darling, D., Barber, L., Afzali, B., . . . Mufti, G. J. (2007). CD4+CD25high Foxp3+ regulatory T cells in myelodysplastic syndrome (MDS). Blood, 110(3), 847-850.

Kyaw, M. H., Rose, C. E.,Jr, Fry, A. M., Singleton, J. A., Moore, Z., Zell, E. R., . . . Active Bacterial Core Surveillance Program of the Emerging Infections Program Network. (2005). The influence of chronic illnesses on the incidence of invasive pneumococcal disease in adults. The Journal of Infectious Diseases, 192(3), 377-386.

Le Floc'h, N., Otten, W., & Merlot, E. (2011). Tryptophan metabolism, from nutrition to potential therapeutic applications. Amino Acids, 41(5), 1195-1205.

Le Polain de Waroux, O., Flasche, S., Prieto-Merino, D., & Edmunds, W. J. (2014). Age-dependent prevalence of nasopharyngeal carriage of streptococcus pneumoniae before conjugate vaccine introduction: A prediction model based on a meta-analysis. PloS One, 9(1), e86136.

Lexau, C. A., Lynfield, R., Danila, R., Pilishvili, T., Facklam, R., Farley, M. M., . . . Active Bacterial Core Surveillance Team. (2005). Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. Jama, 294(16), 2043-2051.

Linet, M. S., Schubauer-Berigan, M. K., Weisenburger, D. D., Richardson, D. B., Landgren, O., Blair, A., . . . Dores, G. M. (2007). Chronic lymphocytic leukaemia: An overview of aetiology in light of recent developments in classification and pathogenesis. British Journal of Haematology, 139(5), 672-686.

Luján, M., Burgos, J., Gallego, M., Falco, V., Bermudo, G., Planes, A., . . . Rello, J. (2013). Effects of immunocompromise and comorbidities on pneumococcal serotypes causing invasive respiratory infection in adults: Implications for vaccine strategies. Clinical Infectious Diseases, 57(12), 1722-1730.

Mackus, W. J., Frakking, F. N., Grummels, A., Gamadia, L. E., De Bree, G. J., Hamann, D., . . . Van Oers, M. H. (2003). Expansion of CMV-specific CD8+CD45RA+CD27- T cells in B-cell chronic lymphocytic leukemia. Blood, 102(3), 1057-1063.

Marshall, N. A., Christie, L. E., Munro, L. R., Culligan, D. J., Johnston, P. W., Barker, R. N., & Vickers, M. A. (2004). Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. Blood, 103(5), 1755-1762.

Martínez-Trillos, A., Pinyol, M., Navarro, A., Aymerich, M., Jares, P., Juan, M., . . . Villamor, N. (2014). Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome. Blood, 123(24), 3790-3796.

Maschmeyer, G., De Greef, J., Mellinghoff, S. C., Nosari, A., Thiebaut-Bertrand, A., Bergeron, A., . . . European Conference on Infections in Leukemia (ECIL). (2019). Infections associated with immunotherapeutic and molecular targeted agents in hematology and oncology. A position paper by the European Conference on Infections in Leukemia (ECIL). Leukemia, 33(4), 844-862.

McFetridge, R., Meulen, A. S., Folkerth, S. D., Hoekstra, J. A., Dallas, M., Hoover, P. A., . . . Musey, L. K. (2015). Safety, tolerability, and immunogenicity of 15-valent pneumococcal conjugate vaccine in healthy adults. Vaccine, 33(24), 2793-2799.

Meisel, R., Toschke, A. M., Heiligensetzer, C., Dilloo, D., Laws, H. J., & von Kries, R. (2007). Increased risk for invasive pneumococcal diseases in children with acute lymphoblastic leukaemia. British Journal of Haematology, 137(5), 457-460.

Mellor, A. L., Lemos, H., & Huang, L. (2017). Indoleamine 2,3-dioxygenase and tolerance: Where are we now? Frontiers in Immunology, 8, 1360.

Mellor, A. L., & Munn, D. H. (1999). Tryptophan catabolism and T-cell tolerance: Immunosuppression by starvation? Immunology Today, 20(10), 469-473.

Mikulska, M., Cesaro, S., de Lavallade, H., Di Blasi, R., Einarsdottir, S., Gallo, G., . . . European Conference on Infections in Leukaemia group. (2019). Vaccination of patients with haematological malignancies who did not have transplantations: Guidelines from the 2017 European Conference on Infections in Leukaemia (ECIL 7). The Lancet.Infectious Diseases, 19(6), e188-e199.

Moore, M. R., Link-Gelles, R., Schaffner, W., Lynfield, R., Lexau, C., Bennett, N. M., . . . Whitney, C. G. (2015). Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: Analysis of multisite, population-based surveillance. The Lancet Infectious Diseases, 15(3), 301-309.

Morrison, V. A. (2010). Infectious complications of chronic lymphocytic leukaemia: Pathogenesis, spectrum of infection, preventive approaches. Best Practice & Research Clinical Haematology, 23(1), 145-153.

Mufson, M. A., Hao, J. B., Stanek, R. J., & Norton, N. B. (2012). Clinical features of patients with recurrent invasive Streptococcus pneumoniae disease. The American Journal of the Medical Sciences, 343(4), 303-309.

Munn, D. H., & Mellor, A. L. (2016). IDO in the tumor microenvironment: Inflammation, counter-regulation, and tolerance. Trends in Immunology, 37(3), 193-207.

Munn, D. H., Shafizadeh, E., Attwood, J. T., Bondarev, I., Pashine, A., & Mellor, A. L. (1999). Inhibition of T cell proliferation by macrophage tryptophan catabolism. The Journal of Experimental Medicine, 189(9), 1363-1372.

Najfeld, V., Menninger, J., Muhleman, D., Comings, D. E., & Gupta, S. L. (1993). Localization of indoleamine 2,3-dioxygenase gene (INDO) to chromosome 8p12-->p11 by fluorescent in situ hybridization. Cytogenetics and Cell Genetics, 64(3-4), 231-232.

Naucler, P., Galanis, I., Morfeldt, E., Darenberg, J., Ortqvist, A., & Henriques-Normark, B. (2017). Comparison of the impact of pneumococcal conjugate vaccine 10 or pneumococcal conjugate vaccine 13 on invasive pneumococcal disease in equivalent populations. Clinical Infectious Diseases, 65(11), 1780-1789.

Ndure, J., Noho-Konteh, F., Adetifa, J. U., Cox, M., Barker, F., Le, M. T., . . . Flanagan, K. L. (2017). Negative correlation between circulating CD4(+)FOXP3(+)CD127(-) regulatory T cells and subsequent antibody responses to infant measles vaccine but not diphtheria-tetanus-pertussis vaccine implies a regulatory role. Frontiers in Immunology, 8, 921.

O'Brien, S., Furman, R. R., Coutre, S., Flinn, I. W., Burger, J. A., Blum, K., . . . Byrd, J. C. (2018). Single-agent ibrutinib in treatment-naive and relapsed/refractory chronic lymphocytic leukemia: A 5-year experience. Blood, 131(17), 1910-1919.

Okasha, O., Rinta-Kokko, H., Palmu, A. A., Ruokokoski, E., Jokinen, J., & Nuorti, J. P. (2018). Population-level impact of infant 10-valent pneumococcal conjugate vaccination on adult pneumonia hospitalisations in Finland. Thorax, 73(3), 262-269.

Papadatou, I., & Spoulou, V. (2016). Pneumococcal vaccination in high-risk individuals: Are we doing it right? Clinical and Vaccine Immunology : CVI, 23(5), 388-395.

Parikh, S. A. (2018). Chronic lymphocytic leukemia treatment algorithm 2018. Blood Cancer Journal, 8(10), 93-018-0131-2.

Pasiarski, M., Rolinski, J., Grywalska, E., Stelmach-Goldys, A., Korona-Glowniak, I., Gozdz, S., . . . Malm, A. (2014). Antibody and plasmablast response to 13-valent pneumococcal conjugate vaccine in chronic lymphocytic leukemia patients--preliminary report. PloS One, 9(12), e114966.

Pérez-Trallero, E., Marimon, J. M., Ercibengoa, M., Vicente, D., & Perez-Yarza, E. G. (2009). Invasive streptococcus pneumoniae infections in children and older adults in the north of Spain before and after the introduction of the heptavalent pneumococcal conjugate vaccine. European Journal of Clinical Microbiology & Infectious Diseases, 28(7), 731-738.

Pichichero, M. E. (2013). Protein carriers of conjugate vaccines: Characteristics, development, and clinical trials. Human Vaccines & Immunotherapeutics, 9(12), 2505-2523.

Pourgheysari, B., Khan, N., Best, D., Bruton, R., Nayak, L., & Moss, P. A. (2007). The cytomegalovirus-specific CD4+ T-cell response expands with age and markedly alters the CD4+ T-cell repertoire. Journal of Virology, 81(14), 7759-7765.

Quesada, V., Conde, L., Villamor, N., Ordonez, G. R., Jares, P., Bassaganyas, L., . . . Lopez-Otin, C. (2011). Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nature Genetics, 44(1), 47-52.

Quiroga, M. P., Balakrishnan, K., Kurtova, A. V., Sivina, M., Keating, M. J., Wierda, W. G., . . . Burger, J. A. (2009). B-cell antigen receptor signaling enhances chronic lymphocytic leukemia cell migration and survival: Specific targeting with a novel spleen tyrosine kinase inhibitor, R406. Blood, 114(5), 1029-1037.

Rashid, H., Khandaker, G., & Booy, R. (2012). Vaccination and herd immunity: What more do we know? Current Opinion in Infectious Diseases, 25(3), 243-249.

Ravandi, F., & O'Brien, S. (2006). Immune defects in patients with chronic lymphocytic leukemia. Cancer Immunology, Immunotherapy : CII, 55(2), 197-209.

Rawstron, A. C., Kreuzer, K. A., Soosapilla, A., Spacek, M., Stehlikova, O., Gambell, P., . . . Montserrat, E. (2018). Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry.Part B, Clinical Cytometry, 94(1), 121-128.

Reinert, R. R., Haupts, S., van der Linden, M., Heeg, C., Cil, M. Y., Al-Lahham, A., & Fedson, D. S. (2005). Invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany, 2001-2003. Clinical Microbiology and Infection, 11(12), 985-991.

Ricciuti, B., Leonardi, G. C., Puccetti, P., Fallarino, F., Bianconi, V., Sahebkar, A., . . . Pirro, M. (2018). Targeting indoleamine-2,3-dioxygenase in cancer: Scientific rationale and clinical evidence. Pharmacology & Therapeutics, doi:S0163-7258(18)30220-1 [pii]

Ringshausen, I., Schneller, F., Bogner, C., Hipp, S., Duyster, J., Peschel, C., & Decker, T. (2002). Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: Association with protein kinase Cdelta. Blood, 100(10), 3741-3748.

Robbins, J. B., Austrian, R., Lee, C. J., Rastogi, S. C., Schiffman, G., Henrichsen, J., . . . Tiesjema, R. H. (1983). Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. The Journal of Infectious Diseases, 148(6), 1136-1159.

Robinson, C. M., Shirey, K. A., & Carlin, J. M. (2003). Synergistic transcriptional activation of indoleamine dioxygenase by IFN-gamma and tumor necrosis factor-alpha. Journal of Interferon & Cytokine Research, 23(8), 413-421.

Rodgers, G. L., & Klugman, K. P. (2011). The future of pneumococcal disease prevention. Vaccine, 29 Suppl 3, C43-8.

Rossi, D., & Gaidano, G. (2016). The clinical implications of gene mutations in chronic lymphocytic leukaemia. British Journal of Cancer, 114(8), 849-854.

Rossi, D., Rasi, S., Fabbri, G., Spina, V., Fangazio, M., Forconi, F., . . . Gaidano, G. (2012). Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. Blood, 119(2), 521-529.

Ruutu, P., Kuusi, M., Nuorti, PJ., & Koskinen, S. (2004). Communicable diseases. In: Aromaa A, Koskinen S, editors. Health and functional capacity in Finland: Baseline results of the Health 2000 examination survey. Publications of the National Public Health Institute, 67-69.

Safdar, A., Rodriguez, G. H., Rueda, A. M., Wierda, W. G., Ferrajoli, A., Musher, D. M., . . . Keating, M. J. (2008). Multiple-dose granulocyte-macrophage-colony-stimulating factor plus 23-valent polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia: A prospective, randomized trial of safety and immunogenicity. Cancer, 113(2), 383-387.

Savage, D. G., Lindenbaum, J., & Garrett, T. J. (1982). Biphasic pattern of bacterial infection in multiple myeloma. Annals of Internal Medicine, 96(1), 47-50.

Schafer, C. C., Wang, Y., Hough, K. P., Sawant, A., Grant, S. C., Thannickal, V. J., . . . Deshane, J. S. (2016). Indoleamine 2,3-dioxygenase regulates anti-tumor immunity in lung cancer by metabolic reprogramming of immune cells in the tumor microenvironment. Oncotarget, 7(46), 75407-75424.

Schlesinger, M., Broman, I., & Lugassy, G. (1996). The complement system is defective in chronic lymphatic leukemia patients and in their healthy relatives. Leukemia, 10(9), 1509-1513.

Simell, B., Lahdenkari, M., Reunanen, A., Käyhty, H., & Väkeväinen, M. (2008). Effects of ageing and gender on naturally acquired antibodies to pneumococcal capsular polysaccharides and virulence-associated proteins. Clinical and Vaccine Immunology, 15(9), 1391-1397.

Sinisalo, M., Aittoniemi, J., Koski, T., Tobin, G., Thunberg, U., Sundström, C., . . . Vilpo, J. (2004). Similar humoral immunity parameters in chronic lymphocytic leukemia patients independent of VH gene mutation status. Leukemia & Lymphoma, 45(12), 2451-2454.

Sinisalo, M., Aittoniemi, J., Oivanen, P., Käyhty, H., Ölander, R., & Vilpo, J. (2001). Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. British Journal of Haematology, 114(1), 107-110.

Sinisalo, M., Vilpo, J., Itälä, M., Väkeväinen, M., Taurio, J., & Aittoniemi, J. (2007). Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine, 26(1), 82-87.

Sorensen, R. U., & Edgar, J. D. M. (2018). Overview of antibody-mediated immunity to S. pneumoniae: Pneumococcal infections, pneumococcal immunity assessment, and recommendations for IG product evaluation. Transfusion, 58 Suppl 3, 3106-3113.

Sorensen, U. B., & Henrichsen, J. (1984). C-polysaccharide in a pneumococcal vaccine. Acta Pathologica, Microbiologica, & Immunologica Scandinavica.Section C, Immunology, 92(6), 351-356.

Stavrum, A. K., Heiland, I., Schuster, S., Puntervoll, P., & Ziegler, M. (2013). Model of tryptophan metabolism, readily scalable using tissue-specific gene expression data. The Journal of Biological Chemistry, 288(48), 34555-34566.

Svensson, T., Kättström, M., Hammarlund, Y., Roth, D., Andersson, P. O., Svensson, M., . . . Kimby, E. (2018). Pneumococcal conjugate vaccine triggers a better immune response than pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia A randomized study by the Swedish CLL group. Vaccine, 36(25), 3701-3707.

Ten Hacken, E., & Burger, J. A. (2016). Microenvironment interactions and B-cell receptor signaling in chronic lymphocytic leukemia: Implications for disease pathogenesis and treatment. Biochimica & Biophysica Acta, 1863(3), 401-413.

Timens, W., Boes, A., Rozeboom-Uiterwijk, T., & Poppema, S. (1989). Immaturity of the human splenic marginal zone in infancy. possible contribution to the deficient infant immune response. Journal of Immunology, 143(10), 3200-3206.

Van der Linden, M., Falkenhorst, G., Perniciaro, S., & Imohl, M. (2015). Effects of infant pneumococcal conjugate vaccination on serotype distribution in invasive pneumococcal disease among children and adults in Germany. PloS One, 10(7), e0131494.

Van der Velden, A. M., Van Velzen-Blad, H., Claessen, A. M., Van der Griend, R., Oltmans, R., Rijkers, G. T., & Biesma, D. H. (2007). The effect of ranitidine on antibody responses to polysaccharide vaccines in patients with B-cell chronic lymphocytic leukaemia. European Journal of Haematology, 79(1), 47-52.

Verhaegen, J., Flamaing, J., De Backer, W., Delaere, B., Van Herck, K., Surmont, F., . . . Peetermans, W. (2014). Epidemiology and outcome of invasive pneumococcal disease among adults in Belgium, 2009-2011. Euro Surveillance, 19(31), 14-22.

Wang, X., Zheng, J., Liu, J., Yao, J., He, Y., Li, X., . . . Huang, S. (2005). Increased population of CD4(+)CD25(high), regulatory T cells with their higher apoptotic and proliferating status in peripheral blood of acute myeloid leukemia patients. European Journal of Haematology, 75(6), 468-476.

Wong, A., Marrie, T. J., Garg, S., Kellner, J. D., Tyrrell, G. J., & SPAT Group. (2010). Increased risk of invasive pneumococcal disease in haematological and solid-organ malignancies. Epidemiology and Infection, 138(12), 1804-1810.

Woyach, J. A., Ruppert, A. S., Heerema, N. A., Zhao, W., Booth, A. M., Ding, W., . . . Byrd, J. C. (2018). Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. The New England Journal of Medicine, 379(26), 2517-2528.

Yan, H., Dong, M., Liu, X., Shen, Q., He, D., Huang, X., . . . Cai, Z. (2019). Multiple myeloma cell-derived IL-32gamma increases the immunosuppressive function of macrophages by promoting indoleamine 2,3-dioxygenase (IDO) expression. Cancer Letters, 446, 38-48.

Yang, Z. Z., Novak, A. J., Ziesmer, S. C., Witzig, T. E., & Ansell, S. M. (2006). Attenuation of CD8(+) T-cell function by CD4(+)CD25(+) regulatory T cells in B-cell non-hodgkin's lymphoma. Cancer Research, 66(20), 10145-10152.

PUBLICATIONS

PUBLICATION I

Indoleamine 2,3-Dioxygenase Activity and Expression in Patients With Chronic Lymphocytic Leukemia

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Original Study

Indoleamine 2,3-Dioxygenase Activity and Expression in Patients With Chronic Lymphocytic Leukemia

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Abstract

Indoleamine 2,3-dioxygenase (IDO) activity and expression is increased in many hematological malignancies, but has not been previously studied in chronic lymphocytic leukemia (CLL). We determined IDO activity and expression in 49 patients with CLL. We found that IDO activity is increased in CLL. This may have some influence on CLL progression.

Background: Indoleamine 2,3-dioxygenase (IDO) is an enzyme involved in the catabolism of tryptophan, suppressing T-cell activity. IDO activity and expression are increased in many malignant diseases, including hematological malignancies. IDO expression can mediate immunotolerance to tumors. IDO activity and expression have not previously been studied in chronic lymphocytic leukemia (CLL). **Methods:** We measured IDO activity by calculating the kynurenine-tryptophan (kyn-trp) ratio. *IDO* and *IDO2* gene expression was determined by using real-time polymerase chain reaction (PCR). **Results:** In patients with CLL, the serum kyn-trp ratio—reflecting increased IDO activity—was significantly higher compared with controls, but in peripheral blood mononuclear cells (PBMCs)—mainly representing malignant B cells—the expression of genes encoding IDO and IDO2 enzymes was reduced. **Conclusions:** Increased IDO activity in patients with CLL may affect disease progression, although it originates from cells other than malignant B cells.

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Introduction

Chronic lymphocytic leukemia (CLL) is an incurable malignancy of mature B-cell lymphocytes.[1] The disease is associated with complex immunodeficiency, patients evincing defects in B- and T-cell function and antigen presentation as well as hypogammaglobulinemia and poor response to vaccines.[2-5]

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Indoleamine 2,3-dioxygenase (IDO) is a key enzyme in tryptophan metabolism and catalyzes the initial rate-limiting step of tryptophan degradation along the kynurenine pathway.[6] Its activity can thus be estimated indirectly by determining the ratio of its metabolite and substrate (ie, the kynurenine-tryptophan [kyn-trp] ratio). The IDO enzyme is encoded by the gene *INDO*, which is located in the short arm of human chromosome 8 (8p12-p11).[7] IDO2 has recently been reported and it bears structural similarity to IDO and its enzymatic activity, but with lower efficacy.[8] IDO2 is encoded by the gene *INDOL1*, which is located on chromosome 8p12 immediately downstream of the gene *INDO*.[9] IDO induces peripheral immunotolerance and immunosuppression by reducing the local concentration of tryptophan and suppressing T-cell proliferation and activation. IDO is expressed in many cells, including antigen-presenting cells,[10,11] whereas lymphoid cells rarely express it under physiologic conditions.[12] In a recent study in mice, a relationship between B cells and dendritic cells capable of expressing IDO in response to interferons was demonstrated.[13] In human adult T-cell leukemia/lym-

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phoma[14] and acute myeloid leukemia (AML),[15] malignant cells express IDO. Manipulation of IDO expression has recently been observed to be a promising treatment option in inflammatory and autoimmune syndromes, cancer, and transplantation.[12]

The aim of this study was to determine IDO activity and its gene expression in CLL cells. Further, the significance of IDO activity as a possible cause of the immunodeficiency state prevailing in CLL—predisposing to infections and leading to impaired vaccination responses—was assessed.

Materials and Methods

The study population for IDO activity measurements comprised 49 patients with CLL and 24 age- and sex-matched controls from Tampere and Turku University Hospitals who had participated in our pneumococcal vaccine response study. All patients had the B-cell phenotype, and the disease stage according to the Binet classification was A in 39 patients, B in 9 patients, and C in 1 patients. Eleven patients had received chemotherapy.[5] Venous blood samples for IDO measurements had been taken before vaccination. Tryptophan (mmol/L) and kynurenine (μmol/L) concentrations in peripheral blood were measured by reverse-phase high-performance liquid chromatography. Tryptophan was separated with a Shimadzu liquid chromatograph LC-10AD VP (Shimadzu Co, Kyoto, Japan) using a 50-mm BDS Hypersil C18 5-μm column (Thermo Fisher Scientific, Waltham, MA). It was monitored by fluorescence with a Shimadzu RF-10A XL (Shimadzu Co) detector at 266-nm excitation and 366-nm emission wavelengths. Kynurenine was separated with a Hewlett-Packard 1100 liquid chromatograph (Hewlett-Packard Co, Palo Alto, CA) using a Merck LiChroCART 55- to 4150-mm cartridge containing a Purospher STAR RP-18 3-μm column (Merck & Co, Darmstadt, Germany). It was determined by ultraviolet absorption at 360-nm wavelength with a Hewlett-Packard G13144 detector. The kyn-trp (μmol/mmol) ratio was calculated by relating concentrations of kynurenine to tryptophan, allowing estimation of IDO activity. The Mann-Whitney *U* test was applied in comparisons of kynurenine and tryptophan concentrations and the kyn-trp ratio. Correlations were calculated by the Spearman rank correlation test.

INDO (encoding IDO enzyme) and *INDOL1* (IDO2) gene expression was studied in 10 patients with CLL and in 7 controls. The disease stage according to the Binet classification was A in all 10 patients. The blood samples were subjected directly to leukocyte separation with a Ficoll-Paque density gradient (Ficoll-Paque PREMIUM, catalogue No. 17-5442-02, General Electric Healthcare Bio-Sciences, Uppsala, Sweden). The peripheral blood mononuclear cell (PBMC) layer was collected and the cells were suspended in 1 mL of RPMI-1640 medium (catalogue No. R0883, Sigma-Aldrich, St. Louis, MO). Immediately after PBMC separation, traces of erythrocytes were lysed with 10 seconds of H_2O treatment and recovered with 0.9% NaCl. Total RNA extraction was performed with the Qiagen RNeasy Midi Kit (catalogue No. 75144, Qiagen, Valencia, CA). The RNA (500 ng) was converted to cDNA using the High Capacity cDNA Reverse Transcription Kit (catalogue No. 4368814, Applied Biosystems, Carlsbad, CA). The isolated RNA was quantified spectrophotometrically (NanoDrop, Thermo Scientific, Waltham, MA) and tested for inhibition to achieve the correct RNA load for cDNA conversion. Levels of gene transcripts were determined by TaqMan real-time PCR. We analyzed the levels of the IDO and IDO2 transcript with a separate single *IDO* gene expression assay (catalogue No. Hs00158027_m1, Applied Biosystems) and an *IDO2* gene expression assay (catalogue No. Hs00401201_m1, Applied Biosystems) and TaqMan real-time PCR. The gene expression results were analyzed with relative quantification (RQ) documents and the RQ Manager software for automated data analysis (Applied Biosystems). The endogenous control for transcript was β-actin (BACT, catalogue No. Hs03023880_g1). The calculations were based on average (ΔCt) values obtained by subtracting the cycle threshold (Ct) value of the endogenous control from that of the given target gene. Next, the $\Delta\Delta$Ct values were calculated by subtracting the ΔCt of the calibrator from the Ct value of both target genes. The RQ value, which designates the fold change for both targets, was derived from the formula: $RQ = 2^{-\Delta\Delta Ct}$. A gene that was regulated > 2-fold (≥ 2.0 or ≤ 0.5) was considered to be significantly regulated.[16]

Table 1 Kynurenine and Tryptophan Concentrations and Their Ratio (kyn-trp) and *INDO* and *INDOL1 (IDO2)* Gene RNA Expression in PBMCs in Patients With CLL and Controls[a]

Character	CLL n = 49	Controls n = 24	*P* Value
Kyn-Trp Ratio, μmol/mmol	37 (31-48)	33 (29-38)	.027
Kynurenine, μmol/L	3.6 (2.7-4.6)	3.1 (2.6-3.8)	.260
Tryptophan, mmol/L	0.094 (0.083-0.107)	0.095 (0.083-0.112)	.425
	n = 10	n = 7	RQ
INDO (IDO), av ΔCt	14.18	11.63	.17
INDOL1 (IDO2), av ΔCt	15.36	12.26	.12

Abbreviations: av ΔCt = average (mean) Δ threshold cycles; CLL = chronic lymphocytic leukemia; IDO = indoleamine 2,3-dioxygenase; PBMCs = peripheral blood mononuclear cells; RQ = relative quantification.
[a] Results Are expressed in medians (quartiles) if not otherwise stated.

Results and Discussion

Kynurenine and tryptophan concentrations and their ratio (kyn-trp) and *INDO (IDO)* and *INDOL1 (IDO2)* gene RNA expressions in PMBCs in patients with CLL and controls are shown in Table 1. The kyn-trp ratio, reflecting increased IDO activity, was significantly higher in patients with CLL than in controls. However, no statistically significant difference in kynurenine or tryptophan concentrations was detected between patients and controls.

The association of the kyn-trp ratio was calculated against several demographic and disease-associated parameters in patients with CLL, including age; sex; disease duration; Binet class; IgG, IgM, and IgA subclasses; blood cell counts (lymphocytes, neutrophils, thrombocytes); and hemoglobin and C-reactive protein levels. The cytomegalovirus (CMV) status or known prognostic markers were not available. A statistically significant association was detected only with age ($r = .367$; $P = .010$; in controls, $P = .949$) and disease duration ($r = .290$; $P = .044$), but not, eg, with Binet class (A vs. B or C; $P = .130$). In a previous study,

the level of IDO activity was higher in the plasma of the nonagenarians compared with young controls.[17] We also evaluated the possible effect of IDO activity on the vaccine response to a 7-valent pneumococcal conjugate vaccine.[5] There was no difference in kyn-trp between vaccine responders and nonresponders among patients with CLL, and no significant correlations between the kyn-trp ratio and serotype-specific responses were detected.

In the gene expression section of the study, we observed a significantly reduced expression of the *INDO* (encoding IDO enzyme) and *INDOL1 (IDO2)* genes in PMBCs, mainly representing malignant B cells in patients with CLL (Table 1), which is in contrast to findings of increased IDO expression in some other hematologic malignancies.[14,15] In recent studies, it has been shown that treatment with chemotherapy decreases IDO activity and expression.[18,19] In our study population, the majority had never received any treatment for CLL. In T-cell/histiocyte-rich large B-cell lymphoma, IDO production may be involved in the poor prognosis of the disease in that IDO can render the microenvironment tolerogenic to the tumor cells.[20]Patients with AML and a higher kyn-trp ratio have lower survival.[15] Our findings would indicate that the increased kyn-trp ratio in patients with CLL—reflecting increased IDO activity—originates from cells other than PBMCs or malignant B lymphocytes. Mesenchymal stromal cells could be responsible for increased IDO activity, and this type of cell can also expresses IDO.[21] Increased IDO activity may contribute to disease progression, eg, by suppressing cell-mediated immunity. It also may take part in interactions between CLL and stromal cells in the CLL microenvironment.

IDO-expressing dendritic cells are involved in the generation of $CD4^+CD25^+FoxP3^+$ regulatory T cells, which are critical regulators of adaptive immunity.[22] The regulatory T-cell number is increased in patients with CLL.[23] Whether this increased IDO activity has a role in the promotion of regulatory T cells and in the complex immunodeficiency in CLL—predisposing to infections and leading to impaired vaccination responses—requires further study. According to our findings, the cells responsible for increased IDO activity in CLL are not malignant B cells.

Clinical Practice Points

- The IDO enzyme is expressed in many cancer cells. IDO can contribute to immunotolerance to tumors.
- To our knowledge, this is the first study to report IDO activity and expression in CLL. We found increased IDO activity in patients with CLL.
- In the future, IDO inhibitors might be effective antitumor agents.

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Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. *N Engl J Med* 2005; 352:804-15.
2. Caligaris-Cappio F, Hamblin TJ. B-cell chronic lymphocytic leukemia: a bird of a different feather. *J Clin Oncol* 1999; 17:399-408.
3. Messmer D, Telusma G, Wasil T, et al. Dendritic cells from chronic lymphocytic leukemia patients are normal regardless of Ig V gene mutation status. *Mol Med* 2004; 10:96-103.
4. Sinisalo M, Aittoniemi J, Käyhty H, et al. Vaccination against infections in chronic lymphocytic leukemia. *Leuk Lymphoma* 2003; 44:649-52.
5. Sinisalo M, Vilpo J, Itälä M, et al. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. *Vaccine* 2007; 26:82-7.
6. Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? *Immunol Today* 1999; 20:469-73.
7. Najfeld V, Menninger J, Muhleman D, et al. Localization of indoleamine 2,3-dioxygenase gene (INDO) to chromosome 8p12–>p11 by fluorescent in situ hybridization. *Cytogenet Cell Genet* 1993; 64:231-2.
8. Ball HJ, Sanchez-Perez A, Weiser S, et al. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. *Gene* 2007; 396:203-13.
9. Ball HJ, Yuasa HJ, Austin CJ, et al. Indoleamine 2,3-dioxygenase-2; a new enzyme in the kynurenine pathway. *Int J Biochem Cell Biol* 2009; 41:467-71.
10. Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and tumor-induced tolerance. *J Clin Invest* 2007; 117:1147-54.
11. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004; 4:762-74.
12. Kahler DJ, Mellor AL. T cell regulatory plasmacytoid dendritic cells expressing indoleamine 2,3 dioxygenase. *Handb Exp Pharmacol* 2009; 188:165-96.
13. Johnson BA 3rd, Kahler DJ, Baban B, et al. B-lymphoid cells with attributes of dendritic cells regulate T cells via indoleamine 2,3-dioxygenase. *Proc Natl Acad Sci U S A* 2010; 107:10644-8.
14. Hoshi M, Ito H, Fujigaki H, et al. Indoleamine 2,3-dioxygenase is highly expressed in human adult T-cell leukemia/lymphoma and chemotherapy changes tryptophan catabolism in serum and reduced activity. *Leuk Res* 2009; 33:39-45.
15. Corm S, Berthon C, Imbenotte M, et al. Indoleamine 2,3-dioxygenase activity of acute myeloid leukemia cells can be measured from patients' sera by HPLC and is inducible by IFN-gamma. *Leuk Res* 2009; 33:490-4.
16. Jylhävä J, Eklund C, Jylhä M, et al. Expression profiling of immune-associated genes in peripheral blood mononuclear cells reveals baseline differences in co-stimulatory signalling between nonagenarians and younger controls: the vitality 90+ study. *Biogerontology* 2010; 11:671-7.
17. Pertovaara M, Raitala A, Lehtimäki T, et al. Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. *Mech Ageing Dev* 2006; 127:497-9.
18. Cavia-Saiz M, Muñiz P, De Santiago R, et al. Changes in the levels of thioredoxin and indoleamine-2,3-dioxygenase activity in plasma of patients with colorectal cancer treated with chemotherapy. *Biochem Cell Biol* 2012; 90:173-8.
19. Sim SH, Ahn YO, Yoon J, et al. Influence of chemotherapy on nitric oxide synthase, indole-amine-2,3-dioxygenase and CD124 expression in granulocytes and monocytes of non-small cell lung cancer. *Cancer Sci* 2012; 103:155-60.
20. Van Loo P, Tousseyn T, Vanhentenrijk V, et al. T-cell/histiocyte-rich large B-cell lymphoma shows transcriptional features suggestive of a tolerogenic host immune response. *Haematologica* 2010; 95:440-8.
21. Sioud M. New insights into mesenchymal stromal cell-mediated T-cell suppression through galectins. *Scand J Immunol* 2011; 73:79-84.
22. Park MJ, Min SY, Park KS, et al. Indoleamine 2,3-dioxygenase-expressing dendritic cells are involved in the generation of CD4+CD25+regulatory T cells in Peyer's patches in an orally tolerized, collagen-induced arthritis mouse model. *Arthritis Res Ther* 2008; 10:R11. Epub 2008 Jan 25.
23. D'Arena G, Laurenti L, Minervini MM, et al. Regulatory T-cell number is increased in chronic lymphocytic leukemia patients and correlates with progressive disease. *Leuk Res* 2011; 35:363-8.

PUBLICATION
II

Invasive pneumococcal disease in patients with haematological malignancies before routine use of conjugate vaccines in Finland

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BRIEF REPORT

Invasive pneumococcal disease in patients with haematological malignancies before routine use of conjugate vaccines in Finland

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ABSTRACT

The baseline national invasive pneumococcal disease (IPD) incidence rate, serotype distribution and serotype coverage of pneumococcal vaccines were evaluated in patients with Hodgkin's and non-Hodgkin's lymphomas, myeloma and leukaemia within 1 year after haematological diagnosis during 1995–2002, before introduction of pneumococcal conjugate vaccines. Pneumococcal serotype distribution among these patients was different from serotypes causing IPD in the general population. The serotype coverages of PCV13 and PPSV23 were 57% and 64%, respectively, lower than in the general population. This reflects a higher predisposition to IPD in vaccinated patients with haematological malignancies and possibly less benefit of herd immunity gained with the wide use of pneumococcal conjugate vaccines in the general population. This data will be useful as a baseline for determining the future role of adult PCV vaccination in these patient groups.

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Introduction

Patients with immunocompromising conditions such as haematological malignancies are at the highest risk for invasive pneumococcal disease (IPD)[1] However, only limited population-based data are available concerning incidence and serotype distribution in these patients. According to a previous study, the overall incidence of IPD in adults with haematological malignancies in Finland was estimated to be 5.5/1000 per year, when population at risk in person years were used as denominators for these malignancies. This is 50-times higher than in the background population.[2]

The antibody responses to pneumococcal vaccines are limited in patients with haematological malignancies.[3–6] In a study with chronic lymphocytic leukaemia (CLL) patients, no antibody responses were detected after a single dose of PPSV23 [3], but 24% of the patients achieved a significant response (defined as an at least 2-fold increase and a post-vaccination concentration of at least 0.35 μg/ml) after a single dose of 7-valent pneumococcal conjugate vaccine (PCV7).[4] Furthermore, a retrospective study with myeloma patients showed that, after a single dose of 23-valent pneumococcal polysaccharide vaccine (PPSV23), only 33% of the patients achieved a significant antibody response defined as a geometric mean antibody titer above 25 units or above 40 units with more than one of the six specific antibody titers below 25 units.[5] After allogeneic stem cell transplantation the response rate to three doses of PCV7 was 79%, but the antibody response was transient.[6]

The serotypes included in pneumococcal vaccines were selected on the basis of serotypes causing IPD in children. The pneumococcal conjugate vaccines PCV10 and PCV13 contain 10 and 13 of these serotypes.[7] The investigational 15-valent pneumococcal conjugate vaccine (PCV15) has expanded coverage for serotypes 22F and 33F.[8] PCVs are thought to be more immunogenic depending on serotype or age when compared to PPSV23 and they also prevent IPD through an indirect effect, i.e. herd immunity.[9] Since September 2010 PCV10 has been included in the Finnish childhood vaccination programme with a 2 + 1 schedule. During the first 3 years the PCV10 uptake was estimated at 95% and the overall IPD rate among vaccine-eligible children was reduced by 80%.[10] PCV13 and PPSV23 have recently been included in the Finnish national vaccination programme for patients who have received haematopoietic stem cell transplantation (HSCT).

We evaluated the incidence of IPD, serotype distribution of the pathogens and serotype coverage of PCV7, PCV10, PCV13 and PPSV23 in patients with specific haematological malignancies (Hodgkin's lymphoma, non-Hodgkin's lymphoma, myeloma and leukaemia) within 1 year after haematological diagnosis in a registry-based national study in Finland during 1995–2002, before the start of the routine infant conjugate vaccine programme.

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Materials and methods

All clinical microbiology laboratories in Finland report invasive pneumococcal isolates to the National Infectious Disease Register (NIDR), a population-based laboratory surveillance system. A case of IPD was defined as isolation of *S.pneumoniae* from blood and/or CSF during 1995–2002. *S.pneumoniae* isolates were serotyped at the National Public Health Institute (KTL) reference laboratory by counter-immunoelectrophoresis or latex agglutination using pneumococcal antisera.[11] Pneumococcal serotypes were grouped as follows: 7-valent PCV types (4, 6B, 9V, 14, 18C, 19F, 23F), 10-valent PCV types (adding serotypes 1, 5 and 7F), 13-valent PCV types (adding serotypes 3, 6A and 19A), 23-valent PPSV types (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F) and all other types.

National IPD surveillance data were linked to the Finnish Cancer Registry database [12] to obtain information regarding diagnosis of haematological malignancy (Hodgkin's lymphoma, non-Hodgkin's lymphoma, myeloma and leukaemia) within 1 year before the first positive culture.[2] In addition, ICD (9^{th} and 10^{th} Revision) codes of haematological malignancy for the IPD episode were collected from the National Hospital Discharge Register (HILMO). To maintain the cumulative incidence of haematological malignancies stable, only diagnosed diseases within 1 year before the first episode of IPD were analysed.

Incidence rates of IPD in patients with haematological malignancy including 95% confidence intervals were calculated using person-time of patients with a specific haematological malignancy in the Cancer Registry as a denominator. Data were analysed using SPSS Statistics 21.0 (Chicago, IL).

National registry data use was authorised by the Ministry of Social Affairs and Health, the Finnish Data Protection Authority and the National Research and Development Centre for Welfare and Health.

Results

Among 4357 cases of IPD identified during 1995–2002,[2] a haematological malignancy (Hodgkin's or non-Hodgkin's lymphoma, myeloma or leukaemia) had been diagnosed in 56 (1.3%) cases within 1 year before the IPD episode. From these 56 IPD cases, the isolates were available for serotyping in 47 cases (84%). The median age of these patients was 64 years (range = 9 months–81 years) at the time of IPD and 32 isolates (68%) were from males. In 45 cases (96%), bacteria were isolated from blood and in two cases from CSF (4%).

The overall incidence rate of IPD in patients with haematological malignancy was 3.8 per 1000 person-years (Table 1). Patients with myeloma had the highest rate of IPD (10.9 cases per 1000 person-years). The most common serotypes were 14 in eight IPD episodes, 6B in five, 11A and 4 in three each and 19F in one (Figure 1). Of the isolates, 47% (22/47), 51% (24/47), 57% (27/47) and 64% (30/47) were serotypes included in PCV7, PCV10, PCV13 and PPSV23, respectively; 32% (15/47) of the isolates were not included in either PPSV23 or PCV13. PCV15 would have yielded serotype coverage for 29 cases (62%). Serotype coverage varied with the type of haematological malignancy. In patients with myeloma the serotypes included in PCV13 and PPSV23 covered 12 (60%) and 14 (70%) cases, respectively (Figure 2).

Table 1. Rates of invasive pneumococcal disease (IPD) in patients with haematological malignancies in Finland during 1995–2002 within 1 year of haematological diagnosis.

Malignancy	Cases of specified malignancy (person years)[a]	IPD cases with specified malignancy	Incidence rate/1000 person years (95% CI)
Myeloma	2 393	26	11 (7.4–16)
Leukaemia[b]	4 201	17	4.1 (2.5–6.5)
Non-Hodgkin's lymphoma	7 131	12	1.7 (1.0–3.0)
Hodgkin's lymphoma	1 080	1	0.9 (0.1–6.6)
Total	14 805	56	3.8 (2.9–4.9)

[a]From the Finnish Cancer Registry.
[b]Acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), chronic myeloid leukaemia (CML).

Discussion

Few population-based studies concerning IPD incidence are available in patients with haematological malignancies. Our data indicates that the risk of IPD in these patients was 35-times higher than in the general population in Finland, where the average annual incidence of IPD was 0.11 cases per 1000 persons.[2] The highest rates were seen in patients with myeloma, consistent with a previous report.[13]

Pneumococcal serotype distribution in patients with haematological malignancies before introduction of the infant conjugate vaccine programme was different from serotypes causing IPD among the general population, where the most common serotypes in the general adult population were 4, 14, 3, 7F and 23F and in children serotypes 6B, 14, 19A, 18C and 7F.[11] In a European Bone Marrow Transplantation (EBMT) survey the identified serotypes in IPD following stem cell transplantation were 23F, 6, 14, 9, 11 and 22, but only 33% of isolates had been serotyped.[14] In Spain serotypes 6A, 23F, 11A and 33F were more frequently found in immunocompromised patients. Serotype 6A was independently related to haematological malignancies.[15] All the most common serotypes in our study are included in PPSV23, but serotype 11A is not covered in PCV13. In an earlier study the serotype coverage of PCV13 and PPSV23 in the general population above 18 years was 75% and 83%, respectively,[11] which is 1.3-times higher than in the patients in this study. The serotype coverages in haematological and haematopoietic stem cell transplant patients in France (i.e. PCV13, 84%; PPSV23, 92%) [16] were also 1.5- and 1.4-times higher than in our study, respectively. Thus, the proportion of serotypes which was not covered in PPSV23 and PCV13 was relatively high in our study.

There are some limitations in our study. The data was collected during the years 1995–2002, before the pneumococcal conjugate vaccine era and possible herd immunity from infant PCV vaccination. In the US, rates of IPD in adults with HIV-infection, another important group of immunocompromised patients, decreased from 399 to 298 per 100 000 (25%) 7 years after PCV7 introduction, whereas rates of non-PCV7 serotypes increased from 168 to 269 per 100 000 (60%).[17] In Finland, after PCV10 was introduced into the national

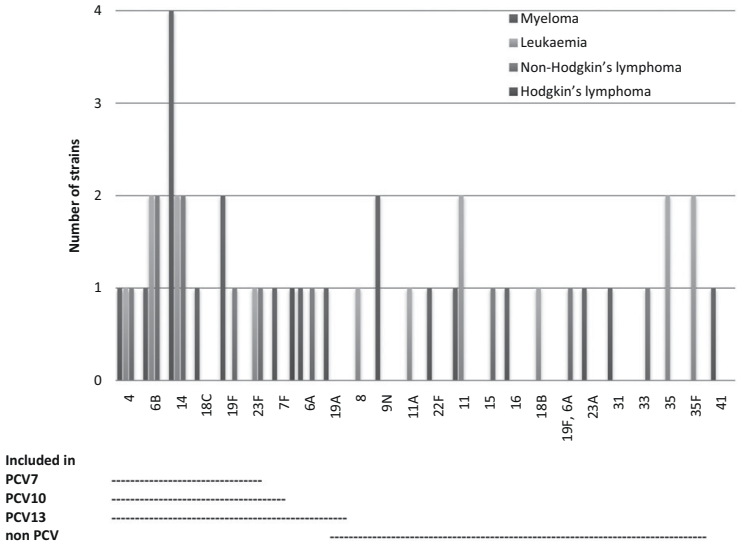

Figure 1. Distribution of pneumococcal serotypes in patients with haematological malignancies in Finland during 1995–2002 within 1 year of diagnosis.

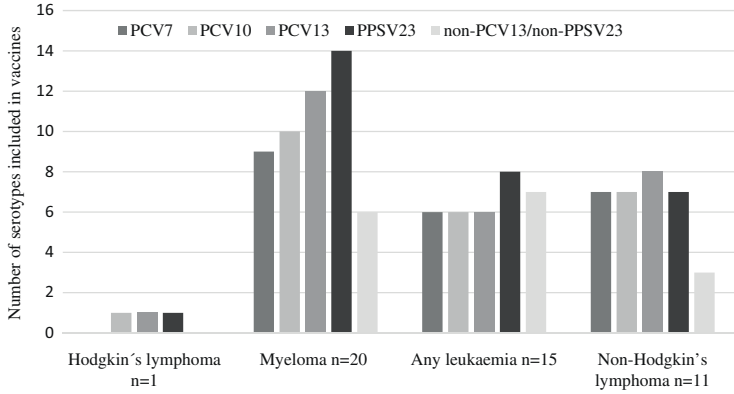

Figure 2. Serotype coverage by pneumococcal vaccines in patients with haematological malignancies in Finland during 1995–2002 within 1 year of diagnosis.

vaccination programme, during the first 3 years non-PCV10 serotypes increased marginally from 0.03 to 0.06 cases per 1000 person-years.[10] Furthermore, the ICD codes collected from the registry and HILMO may have been incomplete and some diagnoses may have been missed, although the Finnish Cancer Registry has a large coverage. Also, the pneumococcal vaccination status was not known, but a national survey in Finland found that, in the high risk groups, the number of patients vaccinated with PPSV23 was low.[18] The number of HSCT recipients was not known, but the proportion of vaccinated HSCT recipients was estimated to be relatively low, because only haematological malignancies diagnosed within 1 year before the first episode of IPD were included in the data.

Our data indicates that the baseline pre-PCV serotype distribution of IPD in patients with haematological malignancies was different from that in the general population. Serotype coverage of both PCV13 and PPSV23 was ~20% lower in this group. These findings suggest that pre-disposition to IPD is still high in vaccinated patients. These data will be useful as a baseline for evaluating the potential indirect effects of the infant PCV10 vaccination programme on patients with haematological malignancies in Finland. They will also be helpful in determining the future role of adult PCV vaccination of these high risk patients. Because of the possible different serotype distribution and lower serotype coverage, the potential benefits from herd immunity may be low and protection of patients with haematological malignancies might require new approaches to vaccination, such as protein-based vaccines [19] and pneumococcal conjugate vaccines with expanded serotype coverage (e.g. PCV15).[8] Further studies during the conjugate vaccine era are warranted to confirm these data.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Kyaw MH, Rose CE , Fry AM, et al. The influence of chronic illnesses on the incidence of invasive pneumococcal disease in adults. J Infect Dis. 2005;192:377–386.

[2] Klemets P, Lyytikäinen O, Ruutu P, et al. Invasive pneumococcal infections among persons with and without underlying medical conditions: implications for prevention strategies. BMC Infect Dis. 2008;8:96.

[3] Sinisalo M, Aittoniemi J, Oivanen P, et al. Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. Br J Haematol. 2001;114:107–110.

[4] Sinisalo M, Vilpo J, Itälä M, et al. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine. 2007;26:82–87.

[5] Hinge M, Ingels HA, Slotved HC, Mølle I. Serologic response to a 23-valent pneumococcal vaccine administered prior to autologous stem cell transplantation in patients with multiple myeloma. APMIS. 2012;120:935–940.

[6] Cordonnier C, Labopin M, Chesnel V, et al. Randomized study of early versus late immunization with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. Clin Infect Dis. 2009;48: 1392–1401.

[7] Rodgers GL, Klugman KP. The future of pneumococcal disease prevention. Vaccine. 2011;29(Suppl 3):S43–S48.

[8] Skinner JM, Indrawati L, Cannon J, et al. Pre-clinical evaluation of a 15-valent pneumococcal conjugate vaccine (PCV15-CRM197) in an infant-rhesus monkey immunogenicity model. Vaccine. 2011;29:8870–8876.

[9] Pulido M, Sorvillo F. Declining invasive pneumococcal disease mortality in the United States, 1990-2005. Vaccine. 2010;28:889–892.

[10] Jokinen J, Rinta-Kokko H, Siira L, et al. Impact of ten-valent pneumococcal conjugate vaccination on invasive pneumococcal disease in Finnish children - a population-based study. PLoS One. 2015;10:e0120290.

[11] Klemets P, Lyytikäinen O, Ruutu P, et al. Trends and geographical variation in invasive pneumococcal infections in Finland. Scand J Infect Dis. 2008;40:621–628.

[12] Brenner H, Hakulinen T. Reduction in selective under-ascertainment bias in population-based estimates of cancer patient survival by age adjustment. Eur J Cancer. 2005;41:1788–1793.

[13] Wong A, Marrie TJ, Garg S, et al. SPAT Group. Increased risk of invasive pneumococcal disease in haematological and solid-organ malignancies. Epidemiol Infect. 2010;138:1804–1810.

[14] Engelhard D, Cordonnier C, Shaw PJ, et al. Early and late invasive pneumococcal infection following stem cell transplantation: a European Bone Marrow Transplantation survey. Br J Haematol. 2002;117:444–450.

[15] Luján M, Burgos J, Gallego M, et al. Effects of immunocompromise and comorbidities on pneumococcal serotypes causing invasive respiratory infection in adults: implication for vaccine strategies. Clin Infect Dis. 2013;57:1722–1730.

[16] Debbache K, Varon E, Hicheri Y, et al. The epidemiology of invasive Streptococcus pneumoniae infections in onco-haematology and haematopoietic stem cell transplant patients in France. Are the serotypes covered by the available anti-pneumococcal vaccines? Clin Microbiol Infect. 2009;15:865–868.

[17] Cohen AL, Harrison LH, Farley MM, et al. Prevention of invasive pneumococcal disease among HIV-infected adults in the era of childhood pneumococcal immunization. AIDS. 2010;2253–2262.

[18] Ruutu P, Kuusi M, Nuorti PJ, Koskinen, S. Communicable diseases. In: Aromaa A, Koskinen S, editors. Health and functional capacity in Finland: baseline results of the Health 2000 examination survey. Helsinki: National Public Health Institute, 2004. pp 67–69.

[19] Olaya-Abril A, Jiménez-Munguía I, Gómez-Gascón L, et al. Identification of potential new protein vaccine candidates through pan-surfomic analysis of pneumococcal clinical isolates from adults. PLoS One. 2013;8:e70365.

PUBLICATION III

Antibody persistence after pneumococcal conjugate vaccination in patients with chronic lymphocytic leukemia

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SHORT REPORT

Antibody persistence after pneumococcal conjugate vaccination in patients with chronic lymphocytic leukemia

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ABSTRACT

Patients with chronic lymphocytic leukemia (CLL) are at a high risk for infections caused by *Streptococcus pneumoniae*. A pneumococcal conjugate vaccine (PCV) can induce a significant antibody response for some CLL patients. In this study we investigated antibody persistence after PCV7 in patients with CLL. The study material comprised 24 patients with CLL and 8 immunocompetent controls. The median antibody concentrations five years after PCV7 were lower for six pneumococcal serotypes in patients with CLL compared to controls, but the difference was not statistically significant. Depending on the serotype, the percentage of the CLL patients with antibody levels suggested to provide protection against invasive pneumococcal disease (IPD) varied from 29 to 71% five years after vaccination. This data suggests that PCV could result in antibody persistence at least five years in CLL patients.

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antibody persistence; chronic lymphocytic leukemia; pneumococcal conjugate vaccine

Chronic lymphocytic leukemia (CLL), a mature B-cell neoplasm, is the most common type of leukemia in adult Caucasians.[1] CLL is associated with a significant dysfunction of the immune system that results in both quantitative and qualitative defects in innate and adaptive immune responses.[2] While hypogammaglobulinemia can occur even in the early stage of disease, it usually becomes more severe during the course of the disease and at more advanced stages.[3] Infections are the most common cause of mortality in CLL patients. The majority of infections are bacterial, caused by common organisms, including *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae*.[4]

Previous studies with the 23-valent pneumococcal polysaccharide vaccine (PPV23) in patients with CLL demonstrated no antibody responses at all or only weak responses.[5–8] In contrast, the immunogenicity of pneumococcal conjugate vaccine (PCV) in patients with CLL has been shown in a few earlier studies. A single dose of 7-valent pneumococcal conjugate vaccine (PCV7) given at an early stage of the CLL resulted in a significant response in almost 40% of patients.[9] Furthermore, 13-valent pneumococcal conjugate vaccine (PCV13) induced at least a two-fold increase in antibody titers from baseline in 58% of previously untreated CLL patients, but an antibody concentration of 0.35 μg/ml was not used as a serological threshold for adequate response.[10] Many current international guidelines recommend PCV13 for immunocompromised patients. The persistence of pneumococcal antibodies in patients with CLL has yet to be studied.

In this follow-up study, pneumococcal antibody persistence was assessed in CLL patients and immunocompetent controls at five years after one dose of PCV7 given as part of an earlier response study.[9]

The study population comprised 24 patients with CLL (12 males and 12 females), with a median age of 64 years (range 47–86 years) from Tampere and Turku University Hospitals. The control population comprised 8 subjects (median age 67 years, range 57–82 years, 4 males and 4 females) without any known immunological or hematological defects from Tampere University Hospital. The patients and control subjects had participated in an earlier pneumococcal vaccine response study with PCV7.[9]

An informed consent to participate was obtained from all patients and controls. The study was approved by the ethical board of the Pirkanmaa Hospital District and the trial was registered at http://ClinicalTrials.gov *(NCT00919321)*.

Clinical and laboratory characteristics of the CLL patients are shown in Table 1. The disease status according to Binet classification was A (early stage of the disease) in 16, B (intermediate) in 2, and C (advanced stage) in 6 patients. A total of 16 patients had never been treated for CLL. Seven patients had suffered from severe infections (needing intravenous antibiotics or hospitalization) and six patients from mild to moderate infections (treated with oral antibiotics) during the five years since PCV7 vaccination. Only one of these infections was pneumococcal infection, i.e. pneumococcal septicemia.

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Table 1. Clinical and laboratory characteristics of the patients with CLL.

Character	Patients with CLL ($N = 24$)
Sex M/F	12/12
Age (years)	64 (47–86)
Binet A/B/C	16/2/6
Past or ongoing therapy	8 (33%)
Lymphocyte count (x10^9/l)	24.3 (0.9–140.0)
Platelet count (x10^9/l)	141 (38–372)
Hemoglobin (g/l)	135 (81–153)
Neutrophil count (x10^9/l)	4.0 (0.6–12.1)
IgG (g/l)	7.4 (3.2–12.5)
IgM (g/l)	0.3 (0.1–5.4)
IgA (g/l)	0.7 (0.2–4.5)

Hypogammaglobulinemia (S-IgG <6.77 g/l) was detected in 11 patients.

The concentrations of serum IgG antibody against pneumococcal capsular polysaccharides were measured by a modification of the 22F inhibition enzyme immunoassay (EIA) method as previously described.[11] An antibody concentration of 0.35 μg/ml was considered as a threshold for protection against invasive pneumococcal disease (IPD), as recommended by World Health Organization WHO.[12] A comparison of antibody concentrations between CLL patients and controls was performed with Fisher's exact test.

Pneumococcal antibody concentrations four weeks and five years after the administration of PCV7 are shown in Table 2. In the CLL patients, median antibody concentrations against pneumococcal serotypes 4, 6B, 18C and 19F after five years were approximately 50% lower than those measured four weeks after vaccination. Furthermore, antibody concentrations against serotypes 9V and 23F were approximately 75% and 65% lower five years after vaccination, respectively. In contrast, the median concentration of the antibody against serotype 14 remained at a similar level over the five-year period following the vaccination. No statistically significant differences were seen in antibody concentrations between CLL patients and controls five years from vaccination. The pneumococcal antibody concentrations in controls declined by more than 50% in each serotype group. The median concentration of the antibody against serotype 9V was 95% lower in controls, as measured five years after the administration of PCV7.

The percentages of subjects whose antibody concentrations were at a level suggested to be protective against IPD five years after the administration of PCV7 are shown in Fig. 1. In patients with CLL, these percentages varied from 29 to 71%, with the lowest proportion found against serotype 4 and the highest against serotypes 18C and 19F. In controls, the corresponding percentages ranged 50- to 87.5%, with the lowest proportion being against serotype 6B and the highest against serotype 18C.

The baseline proportions of suggested protective antibody concentrations before vaccination in patients with CLL ranged 8- to 88%. The lowest baseline proportion was against serotype 4 and the highest against serotype 19F. In controls, the same percentages were 0–100%, with no protective concentrations found against serotype 4. Protective antibody concentrations against serotype 18C were observed in all controls before vaccination.[9]

There is no earlier data available concerning pneumococcal antibody persistence in patients with CLL. In HIV-infected children on HAART, protective antibody concentrations of $\geq$0.5 μg/ml persisted for longer than four years for serotypes 6B and 14 after PCV7-PCV7-PPV23 administration.[13] In adults of 50 years of age and older, antibody concentrations declined over a five-year period following the administration of PCV13 for all 13 serotypes but remained higher than the levels before vaccination, except for serotype 3.[14] Furthermore, in 40% of allogeneic stem cell transplant patients, who received three doses of PCV7 and one dose of PPV23 after transplantation, antibody concentrations remained at a protective level ($\geq$0.50 μg/ml) for 8–11 years.[15]

In our data antibody concentrations in patients with CLL declined during the five years following a single dose of PCV7 for six serotypes and for all serotypes in healthy controls. The number of subjects was quite small, but no statistically significant difference was seen between these two groups in median antibody concentrations. There was, however, a trend toward lower antibody concentrations in CLL patients compared to controls five years after PCV7 administration for all serotypes except for serotype 19F, which was observed in higher concentrations in the patients. Currently recommended PCV13 contains six other serotypes (1, 3, 5, 6A, 7F, 19A), which are not included in PCV7. Antibody persistence in these additional serotypes remains still unclear and warrants further studies.

More than half of the CLL patients remained their antibody concentrations at protective levels for four out of seven serotypes five years after PCV administration, although the median antibody concentrations declined. Most of the patients had

Table 2. Antibody concentrations against pneumococcal antigens of 7-valent conjugate vaccine four weeks and five years after vaccination in patients with CLL and in controls.

	Post-PCV antibody level median (quartiles)		5 yrs post-PCV antibody level median (quartiles)		
Serotype	CLL	Control	CLL	Control	P-value[1]
4	0.30 (0.07–1.22)	1.87 (0.43–6.16)	0.15 (0.02–0.40)	0.52 (0.13–0.90)	0.116
6B	0.55 (0.20–1.82)	0.95 (0.23–14.7)	0.29 (0.09–0.94)	0.39 (0.06–1.24)	1.000
9V	1.40 (0.25–7.12)	18.0 (0.34–63.2)	0.33 (0.15–2.33)	0.97 (0.17–5.06)	0.685
14	0.72 (0.31–2.66)	14.7 (1.01–20.5)	0.73 (0.20–4.01)	2.71 (0.45–5.83)	0.676
18C	1.55 (0.81–5.99)	9.76 (4.65–81.1)	0.73 (0.23–3.06)	1.42 (1.00–2.47)	0.642
19F	2.01 (0.61–9.31)	3.26 (1.20–51.1)	1.07 (0.26–2.88)	0.69 (0.31–1.56)	1.000
23F	1.53 (0.66–13.4)	5.11 (0.98–23.0)	0.51 (0.17–1.56)	1.17 (0.11–2.23)	1.000

[1]Fisher's exact test.

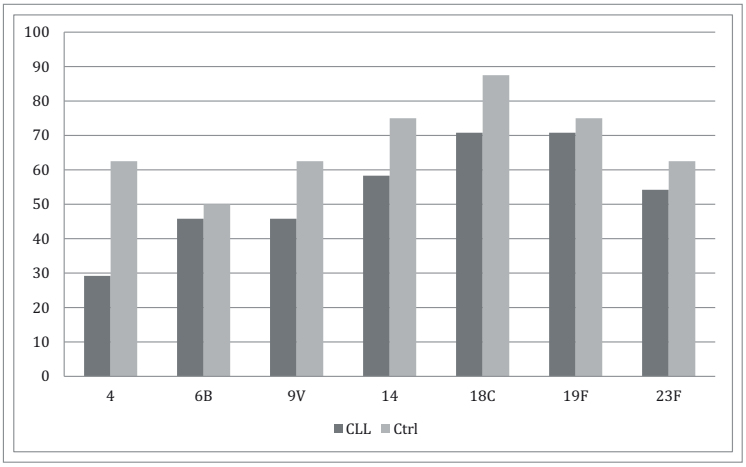

Figure 1. The proportions of antibody concentrations suggestive of protection (≥0.35 μg/ml) against pneumococcal antigens of 7-valent pneumococcal conjugate vaccine in patients with CLL and in controls five years after vaccine administration.

never been treated for CLL, which may have some impact on antibody persistence. Also, the relatively low rates of hypogammaglobulinemia and the low number of severe pneumococcal infections may contribute to better persistence of protection. Although the size of the study population was small, this follow-up data reflects the trend of the antibody persistence five years after PCV7 in patients with CLL.

Earlier data had shown a significant increase in antibody concentrations for all seven antigens in both groups after PCV7 administration.[9] In this follow-up study, the median antibody concentrations in CLL patients at five years after vaccination, as compared with the baseline levels before vaccination, varied depending on the serotype. The post-vaccination antibody concentrations for serotypes 4 and 14 were 1.6- and 1.3 –times higher than the baseline, respectively. In contrast, for serotypes 6B and 18C, the median antibody concentrations declined by almost 20% during the five-year follow-up. Antibody concentrations for the other three serotypes declined to baseline levels. Whether antibody concentrations continue to decline after five years of follow-up remains unclear and warrants a longer follow-up and further studies.

In our earlier data, serotypes 14 and 6B were the most common serotypes causing IPD among patients with hematological malignancy, including CLL. Our finding of favorable persistence for serotype 14 emphasizes the importance of PCV administration for CLL patients in the early stages of the disease, whereas the decline below baseline seen for serotype 6B during the follow-up period may point to a need for a booster dose of PCV or PSV five years after the primary vaccination.

In conclusion, median antibody concentrations in patients with CLL five years after PCV7 administration varied depending on the serotype as compared to controls, and there was a trend toward lower antibody concentrations for six serotypes of PCV7. However, antibody concentrations remained at a level considered to be protective against IPD for four serotypes in more than 50% of the CLL patients. Our findings suggest that PCV given at an early stage of CLL could result in antibody persistence lasting at least five years. The effectiveness and schedule of possible booster immunization needs to be established in future studies.

Disclosure of potential conflicts of interest

The authors declare no conflicts of interest.

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References

1. Linet MS, Schubauer-Berigan MK, Weisenburger DD, Richardson DB, Landgren O, Blair A, Silver S, Field RW, Caldwell G, Hatch M, et al. Chronic lymphocytic leukaemia: An overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol. 2007;139(5):672–686. doi:10.1111/j.1365-2141.2007.06847.x. PMID:18021081.
2. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. ASH Education Program Book. 2008;2008:450–456. doi:10.1182/asheducation-2008.1.450. PMID:19074125.
3. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126(5):573–581. doi:10.1182/blood-2015-03-567388. PMID:26084672.
4. Morrison VA. Infectious complications of chronic lymphocytic leukaemia: Pathogenesis, spectrum of infection, preventive approaches. Best Pract Res Clin Haematol. 2010;23(1):145–153. doi:10.1016/j.beha.2009.12.004. PMID:20620978.
5. Sinisalo M, Aittoniemi J, Oivanen P, Käyhty H, Ölander R, Vilpo J. Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. Br J Haematol. 2001;114(1):107–110. doi:10.1046/j.1365-2141.2001.02882.x. PMID:11472353.
6. Hartkamp A, Mulder AH, Rijkers GT, van Velzen-Blad H, Biesma DH. Antibody responses to pneumococcal and haemophilus vaccinations in patients with B-cell chronic lymphocytic leukaemia. Vaccine. 2001;19(13–14):1671–1677. doi:10.1016/S0264-410X(00)00409-6. PMID:11166890.

7. Safdar A, Rodriguez GH, Rueda AM, Wierda WG, Ferrajoli A, Musher DM, O'Brien S, Koller CA, Bodey GP, Keating MJ. Multiple-dose granulocyte-macrophage-colony-stimulating factor plus 23-valent polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia: A prospective, randomized trial of safety and immunogenicity. Cancer. 2008;113(2):383–387. doi:10.1002/cncr.23561. PMID:18470901.
8. Van der Velden AM, Van Velzen-Blad H, Claessen AM, Van der Griend R, Oltmans R, Rijkers GT, Biesma DH. The effect of ranitidine on antibody responses to polysaccharide vaccines in patients with B-cell chronic lymphocytic leukaemia. Eur J Haematol. 2007;79(1):47–52. doi:10.1111/j.1600-0609.2007.00862.x. PMID:17532765.
9. Sinisalo M, Vilpo J, Itälä M, Väkeväinen M, Taurio J, Aittoniemi J. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine. 2007;26(1):82–87. doi:10.1016/j.vaccine.2007.10.053. PMID:18053620.
10. Pasiarski M, Rolinski J, Grywalska E, Stelmach-Goldys A, Korona-Glowniak I, Gozdz S, Hus I, Malm A. Antibody and plasmablast response to 13-valent pneumococcal conjugate vaccine in chronic lymphocytic leukemia patients-preliminary report. PLoS One. 2014;9(12):e114966. doi:10.1371/journal.pone.0114966. PMID:25506837.
11. Simell B, Lahdenkari M, Reunanen A, Kayhty H, Vakevainen M. Effects of ageing and gender on naturally acquired antibodies to pneumococcal capsular polysaccharides and virulence-associated proteins. Clin Vaccine Immunol. 2008;15(9):1391–1397. doi:10.1128/CVI.00110-08. PMID:18596205.
12. Jodar L, Butler J, Carlone G, Dagan R, Goldblatt D, Kayhty H, Klugman K, Plikaytis B, Siber G, Kohberger R, et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine. 2003;21(23):3265–3272. doi:10.1016/S0264-410X(03)00230-5. PMID:12804857.
13. Abzug MJ, Song LY, Levin MJ, Nachman SA, Borkowsky W, Pelton SI, International Maternal Pediatric Adolescent AIDS Clinical Trials Group P1024 and P1061s Protocol Teams. Antibody persistence and immunologic memory after sequential pneumococcal conjugate and polysaccharide vaccination in HIV-infected children on highly active antiretroviral therapy. Vaccine. 2013;31(42):4782–4790. doi:10.1016/j.vaccine.2013.08.002. PMID:23954381.
14. Frenck RW, Jr., Fiquet A, Gurtman A, van Cleeff M, Davis M, Rubino J, Smith W, Sundaraiyer V, Sidhu M, Emini EA, et al. Immunogenicity and safety of a second administration of 13-valent pneumococcal conjugate vaccine 5 years after initial vaccination in adults 50 years and older. Vaccine. 2016;34(30):3454–3462. doi:10.1016/j.vaccine.2016.04.093. PMID:27155493.
15. Cordonnier C, Labopin M, Robin C, Ribaud P, Cabanne L, Chadelat C, Cesaro S, Ljungman P. Long-term persistence of the immune response to antipneumococcal vaccines after allo-SCT: 10-year follow-up of the EBMT-IDWP01 trial. Bone Marrow Transplant. 2015;50(7):978–983. doi:10.1038/bmt.2015.42. PMID:25867652.

PUBLICATION IV

Antibody response to the 23-valent pneumococcal polysaccharide vaccine after conjugate vaccine in patients with chronic lymphocytic leukemia

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RESEARCH PAPER

Antibody response to the 23-valent pneumococcal polysaccharide vaccine after conjugate vaccine in patients with chronic lymphocytic leukemia

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ABSTRACT

The 23-valent pneumococcal polysaccharide vaccine (PPV23) given alone is ineffective in patients with chronic lymphocytic leukemia (CLL) and better antibody response is achieved with pneumococcal conjugate vaccines (PCVs). In this study, we determine whether CLL patients would achieve a significant antibody response and broaden their serotype coverage against invasive pneumococcal disease (IPD) with PPV23 given five years after the 7-valent conjugate vaccine (PCV7). A total of 24 patients with CLL and eight controls were vaccinated with PPV23 five years after PCV7. Blood samples for evaluation of antibody response to PCV7 serotypes and PPV23 serotypes 5 and 7 were taken before vaccination and one month after it. Post-vaccination samples were available from 20 patients. IgG antibodies were measured with ELISA. Antibody concentrations after PPV23 were significantly lower in CLL patients for six of the PCV7 serotypes and for both PPV23 serotypes. Only 10% to 15% of CLL patients achieved an antibody response suggested to be protective against IPD. Hence, PCV7 given five years before PPV23 did not improve antibody response in patients with CLL. Based on our results, PPV23 given after a PCV primer is not useful against IPD in CLL patients.

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Introduction

Infections are the most important cause of mortality in patients with chronic lymphocytic leukemia (CLL), the most common type of leukemia in adult Caucasians. The usual bacterial pathogens responsible for respiratory tract and urinary tract infections are *Streptococcus pneumoniae, Haemophilus influenzae*, and *Escherichia coli*.[1,2] CLL is associated with an impaired immune system function even at an early stage of the disease.[3] Both the specific and innate immunity are affected, leading to immunosuppression in patients with CLL.[4] Abnormalities in specific (adaptive) immunity include B- and T-cell dysfunction while disorders of the complement system and neutrophil functions represent abnormalities in innate immunity.[1,4–6] While hypogammaglobulinemia can occur at an early stage of CLL, it is usually more severe in the advanced stages of the disease.[7] Low levels of IgG have been observed to correlate with the severity of bacterial infections, morbidity, and mortality.[8]

In contrast to the pneumococcal polysaccharide vaccine (PPV), the T-cell dependent pneumococcal conjugate vaccines (PCVs) have been shown to induce higher avidity antibodies and immunologic memory and to create herd immunity.[9] Antibody responses to pneumococcal vaccines in CLL patients are limited. Most studies with 23-valent PPV (PPV23) have shown no significant antibody responses.[10–13] PCVs, in contrast, have proved immunogenic in CLL patients with limited response in a few earlier studies. In almost 40% of CLL patients, a response, that was considered significant was achieved after one dose of 7-valent PCV (PCV7) given at an early stage of the disease.[14] Furthermore, 13-valent PCV (PCV13) resulted in at least a two-fold increase in antibody titers from the baseline in 58% of untreated CLL patients, compared with 100% for healthy controls.[15] In a recent randomized study, PCV13 was compared to PPV23 in untreated CLL patients. Functional antibody titers measured by opsonophagocytic assay (OPA) were significantly higher for 11 out of 13 serotypes with PCV13 at one month after vaccination and for 6 out of 13 serotypes at six months after vaccination compared to PPV23. All antibody responses for PPV23 were inferior to achieved with PCV13.[16]

PPV23 given after PCV may have a booster effect on antibody responses and may also broaden serotype coverage to PPV23 serotypes in immunocompromised patients. Patients with previously treated Hodgkin lymphoma achieved higher antibody concentrations for six serotypes contained in both vaccines when PPV23 was given one year after PCV7 in contrast to PPV23 alone.[17] In stem cell transplant (SCT) recipients, the antibody response rates to pneumococcal serotypes 1 and 5 not included in PCV7 were 83% after one dose of PPV23 at 12 months and 89% 18 months after 3 doses of PCV7 with the cut-off of ≥0.15 μg/ml set for response.[18]

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Furthermore, 42% of allogeneic SCT recipients, who did not show a response after three doses of PCV7 achieved an antibody response of ≥0.15 μg/ml to the PCV7 antigens after a single dose of PPV23.[19]

In this study, we investigated the effect of a single dose of PPV23 given five years after PCV7 to evaluate whether CLL patients could obtain a boost to their antibody response and broaden their serotype coverage against invasive pneumococcal disease (IPD) with PPV23 primed with PCV7.

Patients and methods

The study population comprised 24 patients with CLL (12 males and 12 females), with a median age of 64 years (range 47–86 years) from Tampere and Turku University Hospitals. The control population comprised eight subjects (median age 67 years, range 57–82 years, four males and four females) without any known immunological or hematological defects from Tampere University Hospital. The patients and control subjects had participated in an earlier pneumococcal conjugate vaccine response study with PCV7 containing capsular polysaccharides (PSs) of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, and in an antibody persistence study.[14,20]

An informed consent to participate was obtained from all patients and controls. The study was approved by the ethical board of the Pirkanmaa Hospital District, and it was conducted in accordance with the Declaration of Helsinki. The trial was registered at *http://ClinicalTrials.gov (NCT00919321).*

The study objectives were to compare antibody concentrations for PCV7 antigens and PPV23 antigens 5 and 7F, as an example for antigens which are not included in PCV7, at one month after vaccination in CLL patients and controls with serotype-specific IgG antibodies as measured by ELISA and to determine the proportions of CLL patients achieving a significant serotype-specific antibody response against IPD after PPV23.

The vaccine used was the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®, MSD), which contains capsular PSs of pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F. A dose of 0.5 ml contains 25 μg of each PS type and phenol as an adjuvant.

Patients and controls received one intramuscular deltoid injection with Pneumovax five years after PCV7. Venous blood samples for antibody analyses were taken before vaccination and one month after it. Serum was separated by centrifugation and stored at −20°C. The concentrations of serum IgG antibody against pneumococcal capsular PSs were measured at the National Institute for Health and Welfare (THL) by a modification of the 22F inhibition enzyme immunoassay (EIA) method as previously described.[21] Additionally, venous blood samples were drawn from CLL patients for the analysis of total blood count and immunoglobulins at the time of vaccination. These were analyzed at a local hospital.

Antibody concentrations and proportionate changes between groups were compared by independent-samples Mann–Whitney test and the significance of antibody responses within groups by two-tailed Fisher´s exact test. The values in the tables are expressed as geometric mean concentrations (GMC) and quartiles. An antibody concentration of 0.35 μg/ml was considered as a threshold for protection against IPD, as recommended by the World Health Organization WHO.[22] A significant antibody response was defined as an at least two-fold increase from the baseline and a post-vaccination level of at least 0.35 μg/ml consistent with earlier PCV7 study.[14]

Results

Among the 24 CLL patients and eight controls enrolled in the study, post-vaccination samples were unavailable in the case of four patients. Therefore, the final study population comprised of 20 CLL patients and eight controls (Table 1). No vaccine-related adverse events were reported. The disease status according to Binet classification was A (early stage of the disease) in 12, B (intermediate) in two, and C (advanced stage) in six patients. A total of 13 patients had never been treated for CLL. After administration of PCV7, six patients had received chemoimmunotherapy or chemotherapy, one patient CD52 monoclonal antibody (alemtuzumab) after chemotherapy and one patient had received allogeneic stem cell transplantation. Hypogammaglobulinemia (S-IgG <6.77 g/l) was detected in nine (45%) patients. Five patients had suffered from severe infections (needing intravenous antibiotics or hospitalization) and five patients from mild to moderate infections (treated with oral antibiotics) during the five years since PCV7 vaccination. Only one of these infections was an invasive pneumococcal disease.

Antibody concentrations after PPV23 were significantly higher in controls for four of PCV7 antigens (4, 9V, 14, 18C) and for both PPV23 antigens (Table 2). In contrast, no difference was observed in antibody concentrations for PCV7 antigens 6B, 19F and 23F. Before PPV23, i.e., five years after PCV7, no statistically significant differences were seen in antibody concentrations between patients with CLL and controls.[20]

After PPV23, only 10% to 15% of CLL patients achieved a significant response to PCV7 antigens defined as an at least two-fold increase and a post-vaccination concentration of at least 0.35 μg/ml (Table 3). Among CLL patients, the responders were the same four patients depending on the serotype, and they had never been treated for CLL. These patients were also among the PCV7 responders. For PPV23 antigens 5 and 7, the corresponding percentages for CLL patients were 20% and 15%, respectively. Despite poor antibody response, 30–75% of CLL

Table 1. Clinical and laboratory characteristics of the patients with CLL.

Character	Patients with CLL (n = 20)
Sex M/F	11/9
Age (years)	63 (47–86)
Binet A/B/C	12/2/6
Past CLL therapy	7 (35%)
Lymphocyte count (x10^9/l)	21.9 (0.9–140.0)
Platelet count (x10^9/l)	141 (38–372)
Hemoglobin (g/l)	135 (81–153)
Neutrophil count (x10^9/l	4.0 (0.6–12.1)
IgG (g/l)	7.4 (3.2–12.5)

The values are expressed as medians and ranges.

Table 2. Pre- and post-vaccination antibody concentrations to seven serotypes included in the 7-valent pneumococcal conjugate vaccine (PCV7, bold) and two serotypes included in the 23-valent pneumococcal polysaccharide vaccine (PPV23) in patients with CLL and in controls.

	Pre-vaccination antibody concentration GMC (µg/ml) (quartiles)		Post-vaccination antibody concentration GMC (µg/ml) (quartiles)[a]		
Serotype	CLL (n = 20)	Control (n = 8)	CLL (n = 20)	Control (n = 8)	p-value[a]
4	**0.12 (0.02–0.40)**	**0.34 (0.13–0.90)**	**0.13 (0.02–0.67)**	**1.96 (1.36–2.82)**	**<0.001**
5	0.16 (0.08–0.44)	0.15 (0.08–0.25)	0.22 (0.08–0.64)	2.20 (0.53–5.57)	0.002
6B	**0.46 (0.11–1.69)**	**0.30 (0.06–1.24**	**0.56 (0.14–3.39)**	**2.28 (0.75–9.96)**	**0.063**
7F	0.38 (0.07–1.27)	0.56 (0.31–1.23)	0.55 (0.07–1.83)	6.74 (2.97–13.8)	<0.001
9V	**0.51 (0.23–2.33)**	**0.91 (0.17–5.06)**	**0.62 (0.32–2.29)**	**3.04 (0.58–12.1)**	**0.033**
14	**0.77 (0.20–4.14)**	**1.56 (0.45–5.83)**	**0.96 (0.17–6.20)**	**6.65 (3.00–24.0)**	**0.021**
18C	**0.93 (0.26–3.06)**	**1.55 (1.00–2.47)**	**1.06 (0.27–4.07)**	**6.81 (3.82–15.7)**	**0.010**
19F	**0.97 (0.30–2.88)**	**0.83 (0.31–1.56)**	**1.29 (0.33–4.20)**	**4.18 (1.88–6.05)**	**0.055**
23F	**0.53 (0.14–1.56)**	**0.82 (0.25–2.23**	**0.67 (0.13–4.41)**	**2.29 (2.07–3.32)**	**0.150**

[a]Between post-vaccination concentrations in patients with CLL and controls
[a]Independent-samples Mann–Whitney U-test (statistical significance if $p \leq .05$)

Table 3. Significant response rates and post-vaccination antibody concentrations suggestive of protection (≥0.35 µg/ml) to seven serotypes included in PCV7 (bold) and two serotypes included in PPV23 in patients with CLL and controls.

	Response rate[a]			Post-vaccination concentration ≥0.35 µg/ml	
	CLL	Controls		CLL	Controls
Serotype	n = 20 (%)	n = 8 (%)	p-value[b]	n = 20 (%)	n = 8 (%
4	**2 (10)**	**7 (88)**	**<0.001**	**7 (35)**	**8 (100)**
5	4 (20)	8 (100)	<0.001	6 (30)	8 (100)
6B	**2 (10)**	**6 (75)**	**0.002**	**10 (50)**	**7 (88)**
7F	3 (15)	6 (75)	<0.001	14 (70)	8 (100)
9V	**3 (15)**	**7 (88)**	**0.001**	**13 (65)**	8 (100)
14	**2 (10)**	**7 (88)**	**<0.001**	**12 (60)**	**8 (100)**
18C	**2 (10)**	**6 (75)**	**0.002**	**15 (75)**	**8 (100)**
19F	**3 (15)**	**7 (88)**	**0.001**	**15 (75)**	**8 (100)**
23F	**2 (10)**	**4 (50)**	**0.038**	**13 (65)**	**8 (100)**

[a]Defined as an at least twofold increase and a post-vaccination concentration of at least 0.35 µg/ml
[b]Fisher´s exact test

patients remained their antibody concentrations at a level ≥0.35 µg/ml suggested to be protective against IPD depending on serotype. In controls, 75–88% achieved a significant response to PCV7 antigens 4, 6B, 9V, 14, 18C, 19F. In contrast, the corresponding rate for serotype 23F was only 50%. For PPV23 antigens, all controls achieved a significant response.

Discussion

According to a few earlier studies, patients with Hodgkin´s lymphoma and SCT recipients benefit from a PCV primer before PPV23.[17–19] Furthermore, in HIV-infected adults, PPV23 given one year after PCV13 improved PCV13 immunogenicity but in contrast, had a negative effect on immunological memory induced by PCV13 by reducing the amounts of memory B-cells.[23] Similar findings have been reported where PPV23 given after PCV depletes immunological memory. Longer intervals between pneumococcal vaccinations diminish this hyporesponsiveness and are beneficial for immunogenicity.[24] Our study was designed based on a long interval between vaccinations consistent with an updated recommendation.[25] However, we did not observe this booster effect in CLL patients. Furthermore, a majority of the untreated CLL patients did not achieve an antibody response considered as significant for PPV23. Hence, in our data CLL therapy after PCV7 did not seem to have an influence on poor antibody responses for PPV23.

An antibody concentration of 1.3 µg/ml is recommended as a cut-off for protective antibody level against IPD in primary immunodeficiencies.[26] This higher cut-off level may also be extrapolated to acquired immunodeficiencies, such as CLL related immune dysfunction. In our data, all responders in CLL group achieved this higher antibody concentration expect two out of four CLL patients for antibody 5.

This is the first study to evaluate the effect of the 23-valent polysaccharide vaccine after the conjugate vaccine (PCV7) in patients with CLL. Although ≥50% of CLL patients remained their antibody concentrations at a protective level for 7 out of 9 serotypes, most of the CLL patients did not achieve an antibody response considered to be significant, as compared to controls. These data suggest that PCV is not capable of inducing memory B-cells in CLL patients, although our earlier data showed antibody persistence at least five years after PCV7, depending on the serotype.[26] One probable reason for a poor antibody response with PPV23 after the PCV primer is abnormalities in both B- and T – cell functions even at an early stage of CLL, leading to inadequate immunogenicity of conjugate vaccines.[1,4,15] Stage of the disease in our data did not impair antibody responses as most of the patients were Binet A patients.

Our study has some limitations. The sample size remained small due to the natural course of CLL and the five-year interval between vaccinations. PCV7 has also been replaced with PCV13, but both have the same carrier protein, CRM.[9] Furthermore, PCV13 has also been shown to generate suboptimal responses in CLL patients.[15,16] Hence, our data should reflect immune responses to both PCV7 and PCV13.

A PPV23 booster dose given five years after PCV7 seemed to be ineffective in inducing an antibody response suggested to be protective against IPD in CLL patients. Based on these data, PPV23 may not be useful as a part of the vaccination program after PCVs in patients with CLL. Future studies with the PCV13 primer are warranted in order to verify our data. Furthermore, whether CLL patients would benefit from a PCV13 booster instead of PPV23 needs to be studied.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

1. Morrison VA. Infectious complications of chronic lymphocytic leukaemia: pathogenesis, spectrum of infection, preventive approaches. Best Prac Res Clin Haematol. 2010;23:145–53. doi:10.1016/j.beha.2009.12.004.
2. Linet MS, Schubauer-Berigan MK, Weisenburger DD, Richardson DB, Landgren O, Blair A, Silver S, Field RW, Caldwell G, Hatch M, et al. Chronic lymphocytic leukaemia: an overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol. 2007;139:672–86. doi:10.1111/j.1365-2141.2007.06847.x.
3. Moreira J, Rabe KG, Cerhan JR, Kay NE, Wilson JW, Call TG, Leis JF, Jelinek DF, Schwager SM, Bowen DA, et al. Infectious complications among individuals with clinical monoclonal B-cell lymphocytosis (MBL): A cohort study of newly diagnosed cases compared to controls. Leukemia. 2013;27:136–41. doi:10.1038/leu.2012.187.
4. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. ASH Educ Prog Book. 2008;2008:450–56.
5. Schlesinger M, Broman I, Lugassy G. The complement system is defective in chronic lymphatic leukemia patients and in their healthy relatives. Leukemia. 1996;10:1509–13.
6. Kontoyiannis DP, Georgiadou SP, Wierda WG, Wright S, Albert ND, Ferrajoli A, Keating M, Lewis RE. Impaired bactericidal but not fungicidal activity of polymorphonuclear neutrophils in patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2013;54:1730–33. doi:10.3109/10428194.2012.750723.
7. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126:573–81. doi:10.1182/blood-2015-03-567388.
8. Rozman C, Montserrat E, Vinolas N. Serum immunoglobulins in B-chronic lymphocytic leukemia. natural history and prognostic significance. Cancer. 1988;61:279–83.
9. Pichichero ME. Protein carriers of conjugate vaccines: characteristics, development, and clinical trials. Hum Vaccin Immunother. 2013;9:2505–23. doi:10.4161/hv.26109.
10. Sinisalo M, Aittoniemi J, Oivanen P, Käyhty H, Ölander R, Vilpo J. Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. Br J Haematol. 2001;114:107–10.
11. Hartkamp A, Mulder AH, Rijkers GT, van Velzen-Blad H, Biesma DH. Antibody responses to pneumococcal and haemophilus vaccinations in patients with B-cell chronic lymphocytic leukaemia. Vaccine. 2001;19:1671–77.
12. Safdar A, Rodriguez GH, Rueda AM, Wierda WG, Ferrajoli A, Musher DM, O'Brien S, Koller CA, Bodey GP, Keating MJ. Multiple-dose granulocyte-macrophage-colony-stimulating factor plus 23-valent polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia: A prospective, randomized trial of safety and immunogenicity. Cancer. 2008;113:383–87. doi:10.1002/cncr.23561.
13. Van der Velden AM, Van Velzen-Blad H, Claessen AM, Van der Griend R, Oltmans R, Rijkers GT, Biesma DH. The effect of ranitidine on antibody responses to polysaccharide vaccines in patients with B-cell chronic lymphocytic leukaemia. Eur J Haematol. 2007;79:47–52. doi:10.1111/j.1600-0609.2007.00862.x.
14. Sinisalo M, Vilpo J, Itälä M, Väkeväinen M, Taurio J, Aittoniemi J. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine. 2007;26:82–87. doi:10.1016/j.vaccine.2007.10.053.
15. Pasiarski M, Rolinski J, Grywalska E, Stelmach-Goldys A, Korona-Glowniak I, Gozdz S, Hus I, Malm A. Antibody and plasmablast response to 13-valent pneumococcal conjugate vaccine in chronic lymphocytic leukemia patients–preliminary report. PLoS One. 2014;9:e114966. doi:10.1371/journal.pone.0114966.
16. Svensson T, Kättström M, Hammarlund Y, Roth D, Andersson PO, Svensson M, Nilsson I, Rombo L, Cherif H, Kimby E. Pneumococcal conjugate vaccine triggers a better immune response than pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia A randomized study by the swedish CLL group. Vaccine. 2018;36:3701–07. doi:10.1016/j.vaccine.2018.05.012.
17. Chan CY, Molrine DC, George S, Tarbell NJ, Mauch P, Diller L, Shamberger RC, Phillips NR, Goorin A, Ambrosino DM. Pneumococcal conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine after treatment of Hodgkin's disease. J Infect Dis. 1996;173:256–58. doi:10.1093/infdis/173.1.256.
18. Cordonnier C, Labopin M, Chesnel V, Ribaud P, de Camara RL, Martino R, Ullmann AJ, Parkkali T, Locasciulli A, Yakouben K, et al. Immune response to the 23-valent polysaccharide pneumococcal vaccine after the 7-valent conjugate vaccine in allogeneic stem cell transplant recipients: results from the EBMT IDWP01 trial. Vaccine. 2010;28:2730–34. doi:10.1016/j.vaccine.2010.01.025.
19. Cordonnier C, Labopin M, Chesnel V, Ribaud P, De La Camara R, Martino R, Ullmann AJ, Parkkali T, Locasciulli A, Yakouben K, et al. Randomized study of early versus late immunization with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. Clin Infect Dis. 2009;48:1392–401. doi:10.1086/598324.
20. Lindström V, Aittoniemi J, Salmenniemi U, Käyhty H, Huhtala H, Itälä-Remes M, Sinisalo M. Antibody persistence after pneumococcal conjugate vaccination in patients with chronic lymphocytic leukemia. Hum Vaccin Immunother. 2018;14:1471–74. doi:10.1080/21645515.2018.1436424.
21. Simell B, Lahdenkari M, Reunanen A, Käyhty H, Väkeväinen M. Effects of ageing and gender on naturally acquired antibodies to pneumococcal capsular polysaccharides and virulence-associated proteins. Clin Vaccine Immunol. 2008;15:1391–97. doi:10.1128/CVI.00110-08.
22. Jodar L, Butler J, Carlone G, Dagan R, Goldblatt D, Käyhty H, Klugman K, Plikaytis B, Siber G, Kohberger R, et al. . Vaccine. 2003;21:3265–72.
23. Farmaki PF, Chini MC, Mangafas NM, Tzanoudaki MT, Piperi CP, Lazanas MZ, Spoulou VS. Immunogenicity and immunological memory induced by the 13-valent pneumococcal conjugate followed by the 23-valent polysaccharide vaccine in HIV-infected adults. J Infect Dis. 2018;218:26–34. doi:10.1093/infdis/jiy135.
24. Papadatou I, Spoulou V. Pneumococcal vaccination in high-risk individuals: are we doing it right? Clin Vaccine Immunol. 2016;23:388–95. doi:10.1128/CVI.00721-15.
25. Kobayashi M, Bennett NM, Gierke R, Almendares O, Moore MR, Whitney CG, Pilishvili T. Intervals between PCV13 and PPSV23 vaccines: recommendations of the advisory committee on immunization practices (ACIP). MMWR Morb Mortal Wkly Rep. 2015;34:944–47. doi:10.15585/mmwr.mm6434a4.
26. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, Kumararatne D, Harville TO, Hesterberg P, Koleilat M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the basic and clinical immunology interest section of the american academy of allergy, asthma & immunology. J Allergy Clin Immunol. 2012;130:S1–24. doi:10.1016/j.jaci.2012.07.002.